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The Biophysical Properties of the Cervical-Vaginal Secretions

Erik Odeblad et al.

Introduction

THE PURPOSE of this contribution is to report and review some work on the biophysical properties of the human cervical and vaginal secretions and relate these properties to the practical use of natural family planning (NFP). The work reported here has been performed during the past 27 years, first at the Department of Obstetrics and Gynecology of Karolinska Institutet, Sabbatsberg Hospital, Stockholm (11 years) and then at the Department of Medical Biophysics, University of Umeå, Sweden (16 years).

This work has led to a rational classification of the types of mucus normally biosynthesized by the cervical mucosa during the various phases of the menstrual cycle. It has also resulted in a better understanding of the normal biological signs of ovulation, the mucus symptoms and their dependence on the biosynthetic processes in the cervix, as well as mucus convection and degradation in the vagina. The possibility for a woman to recognize her mucus is, therefore, the end result of complicated biological events occurring in the cervico-vaginal system. Finally our studies have also led to increased knowledge about pathological forms of biosynthesis (hypo-, and hyper- and dys-secretion) and their possible role

Erik Odeblad, M.D., Ph.D., is professor and chairman, Department of Medical Biophysics, University of Umeå, Umeå, Sweden and formerly research fellow of the Swedish Medical Research Council, Department of Obstetrics and Gynecology, Karolinska Institutet, Sabbatsberg Hospital, Stockholm, Sweden. **Axel Ingelman-Sundberg, M.D.,** is emeritus professor and chairman, Department

in female subfertility and infertility.

It was recognized as early as the middle of the 19th century that the secretion from the cervix could vary (Pouchet 1847, Smith 1855, Sims 1868) but not much attention was given to these studies. It was not until the beginning of the 1930s that (1) a connection was established between the thin, translucent, sperm-receptive secretion and the probable time of ovulation (Seguy & Simonnet 1933, Seguy & Vimeux 1933) and (2) the role of estrogenic hormones was shown (Werner & Collier 1933). In our own studies, this type of mucus was therefore called type E (Odeblad 1968b) while type G was the mucus secreted during the corpus-luteum phase, being thick, opaque, and not receptive for sperm. In 1976 it became clear that the E secretion was, in fact, a mixture of two distinctly different types of mucus, now called type S and type L (originally E_S or E-S and E_L or E-L), and that the mucus *in vivo* (inside the cervical canal) is a "mosaic" made up of "pieces" of these two types (Odeblad 1977). The pieces of S mucus are long and narrow (S=string), while the L pieces are oval (L=loaf). Even if both are translucent and have only slightly different viscosity and spinnbarkeit, they have quite different biological functions. Both of them must coexist together to bring about maximum fertility, as will become evident from the following presentation.

Our first proposal regarding the biomolecular structure of the cervical mucus was made by Odeblad 1959a. In 1966 the secretory function of single secreting units in the cervix was demonstrated and the iso units (isomucorrhoic glands) were described (Odeblad 1966a, 1966b). A couple of years later the types E and G of cervical mucus were defined (Odeblad 1968b), and after many years of work the E type was divided into the two types now known as S and L (Odeblad 1977). Several papers on these types of mucus and their

of Obstetrics and Gynecology, Karolinska Institutet, Sabbatsberg Hospital, Stockholm, Sweden. The following co-authors are from the Department of Medical Biophysics, University of Umeå, Umeå, Sweden: **Lena Hallström,** physiology technician; **Astrid Höglund,** cytology technician; **Unto Leppänen,** bio-electronics engineer; **Karin Lisspers, B.M.; Eva Perenyi, B.M.,**predoctoral student; **Karin Rudolfsson-Åsberg,** bio-medical engineer; **Karin Sahlin, B.Sc.,**

arrangement in the living cervix have appeared (Höglund & Odeblad 1977, Odeblad 1978, Odeblad et al. 1978, Ingelman-Sundberg & Odeblad 1980). Also other authors have described the S-L-G mucus system (Elstein 1978, Billings & Westmore 1980, Elstein 1982). Our papers on the biophysics of the vagina began in 1959 (Odeblad 1959b) and several others have appeared (Odeblad 1960, 1964, 1965; Rudolfsson-Åsberg & Odeblad 1971b). The question of a sperm-motility activating factor from the isthmus was raised by Odeblad 1975, but has not yet been definitely proven.

Clinical Material

These studies started in 1955 long before the radioimmunoassay (RIA) methods for LH determination were used routinely, and we had to rely on other signs and examination of the ovaries (Dickinson 1933, Hartman 1936) to monitor the cyclic changes occuring in the ovary and during the time of ovulation. To ensure the uniformity of the present material, only women examined in this way according to a special scheme (presented below) are included in this report. From a total research pool of about 2,000 women (700 from Stockholm and 1,300 from Umeå), a total of 172 fertile women (107 from Stockholm and 65 from Umeå) are considered in this paper. In table III, 24 women from Umeå not proven fertile, are also included, so a total of 196 women form the basis for this report. The patients selected fulfill the following criteria:

1. The primary reasons why these women came for examination were the following: health examination (119), mild dysmenorrhea (23), mild premenstrual tension (3), noncomplicated trichomonas vaginitis (11), fertility advice (14), or contraceptive advice (26).
2. The women wanted for various reasons, to have studies on their cervical mucus performed (to check their fertility, to aid in their

predoctoral student; **Carola Lindström-Sjögren,** physiology technician; **Carin Sjöström, B.Sc.,** predoctoral student; **Lena Strandberg-Bergström, B.Sc.,** predoctoral student; **Lisbeth Wikström,** physiology technician; and **Marianne Wikström, B.Sc.** This paper is based on lectures presented at Anahuac University, Mexico City, August 19, 1982 and the Second Congress of the Families of the Americas, August 20-24, 1982, Acapulco, Mexico.

getting pregnant, to understand their own bodies, etc.).

3. Women were not subjected to acute or chronic external stress and no symptoms and signs of stress were evident.
4. The women were fertile as shown by pregnancy before or after the present studies and by the calculation of a fertility index (Bergström-Strandberg & Odeblad 1983). As previously mentioned, 24 women included in table III had not been pregnant.
5. Only cycles between 24 and 35 days long were considered.
6. The subcutanous fat layer was thin enough to permit adequate bimanual examination.
7. Women who could relax their abdominal muscles and otherwise cooperate so that it was possible to follow the cyclic changes in the ovaries during one or several menstrual cycles using bimanual examinations.
8. The uterus was not in retroflexion.
9. The cycle was not disturbed by any serious illness.

Clinical Investigations

The examination scheme includes at least five careful bimanual gynecologic examinations during a menstrual cycle according to the following rules (day 0 = estimated day of ovulation):

Days -8 to -5:	Both ovaries must be palpable and identified as resistances about 0.5 x 1.0 x 1.5 cm large, more or less tender and with slight irradiating pain upon examination.
Day -2 to -1:	A follicle of diameter 1.0 cm or more is felt in one of the ovaries, not present at the preceding examination.
Day 0:	The follicle has grown in size and is about 1.5 cm in diameter. The ovary is very tender.
Day +1:	The follicle has disappeared. The corresponding ovary has become less tender. The borderlines of the ovary are unsharp; it is more difficult to feel the size and shape of the ovary. The abdomen exhibits clear muscular defense or the psoas sign on the same side; and percussion echo is more dampened on the ovulating side.
Day +5 to +9:	A soft, untender resistance 2.0 cm in diameter, presumably a corpus luteum, is felt on the place of the former follicle.

All these examinations had to be performed with extreme skill in order to not damage the ovary and its structures, and permit determination of the time of morphological ovulation within 24 hours or 12 hours depending upon the frequency of examination. Besides the physical signs of ovulation at repeated bimanual examinations of the ovaries, three other independent methods were used to check the ovulatory nature of the cycle:

1. The size of the external uterine os.
2. BBT and/or premenstrual symptoms and/or cytology.
3. The crystallization pattern of cervical mucus.

Occasionally urinary pregnandiol or blood progesterone tests were taken, and they indicated that ovulation had occurred.

ad 1. At all examinations the diameter or size of the external uterine os was recorded, either with conical sound if the opening was round or photographically if the opening was irregular. Also the structure of the paraurethral vaginal pockets was recorded photographically.

ad 2. BBT records were used in many cycles. However, it soon turned out in our material that BBT was not always reliable for many reasons. Some of our patients had irregular daily working hours, causing altered circadian temperature variations. In our country nosocomial infections are common, causing fluctuations in BBT. Also many young women, mainly student nurses and medical students, frequently made weekly travels to their parents' homes, disturbing the possibility of recording BBT. Only 92 cycles had acceptable BBT records.

The women reported the state of their breasts, whether they felt tender or not, and the condition of the breasts were also examined. In this way the diagnosis of premenstrual breast swelling was ascertained. Vaginal cytology with Papanicolau or a special staining with methyl-orange-china-blue (Höglund 1972) was used in about one-third of the cases.

ad 3. The degree of crystallization was studied according to the scheme used by Moghissi et al. (1972).

The investigator or the woman, herself, kept daily records on the cervico-vaginal discharge she experienced, especially the occurrence of mucus or slippery discharge. Some women

collected their discharge on pads which were analyzed in our laboratory. These two methods were often combined. In some cases it could not be excluded that daily or twice daily examinations with removal of the bulk cervical mucus interfered with the discharge. When only single crypt samples were removed, such interference was less likely.

When investigating the patient in the periovulatory period, cervical mucus was often found in the vagina. It was soon discovered that the location of this extracervical mucus might play a fundamental role in the mucus symptoms that the woman experienced. Its intravaginal localization and amount was therefore described, and, when possible, also the character of the mucus. In some instances the mucus pieces were also removed for further investigation by biophysical methods.

Sampling

1. Cervical Samples

The cervical samples removed for biophysical studies were of four kinds: bulk cervical regular samples, cervical minisamples, bulk cervical samples with preserved internal structure, and microsamples from single secretory units.

Bulk cervical regular samples. These samples were removed by gentle suction in a 1.5 mm ID glass tube of the type used for the blood sedimentation test (Westergren tube). If the sample could not be analyzed immediately, the tube was sealed with wax or plastic film in both ends until the analyzing could be done. These tubes were used for most investigations.

Cervical minisamples. These samples were removed in 1.0 mm ID capillary glass tubes which could be used directly for Rayleigh or Raman scattering spectroscopy. The samples could also be transferred to other special tubes for the same purpose.

Bulk cervical samples with preserved structure. This type of sampling could be used only in very few instances. 5 mm ID thin-glass Varian NMR tubes were used; they were carefully cut and the ends smoothed in a gas flame. The tube was inserted in the cervix only in cases where it fit a circular external os exactly and the cervical canal was perfectly cylindrical. A gentle suction

was applied simultaneously with the insertion, "punching out" the cervical mucus plug with a minimum of geometrical distortion. In this way the internal string-loaf structure was retained and could be studied by special optical methods. Only about 80 successful samplings of this kind could be done over a 10 year period.

Microsamples from single secretory units. These were removed with 0.3 - 0.6 mm ID thin-glass capillary tubes under suction, inserted under ocular control in the colposcope. The handling of these samples has been described in detail by Odeblad 1966b. The location of the sampling site was carefully noted as follows:

On the portio, the transformation zone = T, the ectopic mucosal zone = K, and the clockwise notation and distance from the external os center was noted.

In the canal, A = the lowest 1/6 of the canal, B = the next 1/6 (C, D, E, etc.), and F = highest 1/6 of the canal (see fig. 16).

2. *Samples from the Isthmus Region* (I)

These were taken in the same way as the microsamples. E, F, and I samples could usually be obtained only during the periovulatory period.

3. *Vaginal Samples*

Vaginal samples were removed with cotton swabs for cytology and with glass tubes or spoons of stainless steel for other investigations. Samples were obtained from different locations in the vagina: the fornices, the subcervical region, the middle vagina, the lower vagina, or the paraurethral pockets.

Biophysical Investigations on Isthmic, Cervical, and Vaginal Samples

Depending on the amount of material and access to equipment, the following studies were performed:

1. Simple physical observations: (a) the amount of material by weight or volume, (b) optical transparency, usually qualitatively but occasionally by light transmission measurement, (c) percent dry substance by weight.
2. Classical rheologic methods: (a) flow viscosimetry (Odeblad 1968a), (b) spinnbarkeit.
3. Proton NMR on bulk mucus: (a) NMR shift relative to pure water (precision measurement according to Odeblad 1966a), (b) T_1

determination with component resolution (Odeblad 1966b, 1968b), (c) T_2 measurement.
It is important to note that these studies were undertaken at a temperature of +21°C. The reported T_1 values should be multiplied by about 1.40, and the log T_1 values should be increased by +0.15 to be valid at +37°C (body temperature). The viscosity values should be multiplied by 0.71 to correspond to the *in vivo* situation. Approximately the same correlation applies to T_2. The chemical shift relative to water did not need correction.

4. Proton NMR spectroscopy on dehydrated mucus, redissolved in D_2O, using a Varian T60 spectrometer, at +35°C.
5. Micro-proton NMR T_1 measurements (Odeblad 1966b) on crypt mucus and typing as S, L, or G mucus. (See point 3 for temperature correction.)
6. Sperm migration in the slide test (Moghissi et al. 1964).
7. Sperm migration and postcoital tests on "punched-out" whole plugs of midcycle mucus, using direct or television microscopy, combined with biomathematical evaluation (see figs. 8 and 9).
8. Crystallization studies on bulk mucus with identification and (in some cases) quantification of S, L, and G mucus. Criteria: The S mucus shows fine, parallel needles; the L mucus shows flower-like arrangements or large and arborized, palm-leaf-like crystals; the G mucus shows small cubic or irregular crystals or no crystals at all. Recently the S mucus has been subdivided into three subtypes, S1, S2, and S3 (see fig. 22 and the corresponding legend for explanation).
9. ESCA spectroscopy with a Varian IEC 30 spectrometer. A dehydrated mucus sample is irradiated in a vacuum with monochromatic X rays, and the emitted photoelectrons are displayed in an energy spectrum which, in turn, gives the electron binding energy. This gives information on the type of chemical bonding. We have especially studied the Na (sodium) and S (sulfur) regions of the spectra in the various types of mucus.
10. The wet mucus was irradiated with a 633 nm He laser beam and the interference pattern of the scattered light was fed into an autocorrelation unit for translation from frequency to time domain. The method used has been described (for bronchial mucus) by Rosenhall and Odeblad 1980.
11. A Bruker Raman spectrometer RT20 with a triple monochromator system was used. The sample was irradiated with a 442 nm Cd laser beam and the Raman spectrum was recorded in the

Stokes region in the shift region 30 - 8000 cm^{-1}. The A_1 and B_1 stretching bands in the region 2700 - 3700 cm^{-1} were ascribed to water in the sample, and the broad region called β was interpreted as Resonance Raman from carbo- or heterocyclic residues in the proteins and nucleic acids in the sample. See figures 12 and 13 for further comments on the technique and theory.

12. Cell countings and cell distribution studies. The mucus was placed in a Bürker counting chamber and the cells per volume element were counted, partly in native mucus and partly in separated mucus types. It is possible to differentiate between various cell types, but these attempts are not reported here. The cell count is small in S mucus, higher in L mucus, and highest in G mucus. The analysis has no value if cervical inflammatory conditions are present.
13. Electron spin resonance. A Varian E-3 spectrometer was used operating at 9.5 GHz and a field of 2500-4000 gauss. Dehydrated samples were used and placed in quartz tubes for analysis.

Results

A considerable amount of data have been collected and only selected parts can be presented here. The selection of material for the present paper has been undertaken with the aim that it might enlighten the understanding and practical use of NFP methods. Other results will be published in several forthcoming papers. A compilation of important data is given in table II and the results are illustrated in figures 1-23. We will now describe some of the results in more detail.

The Types of Cervical Mucus and Their Composition

Very simple observations that any doctor and also the patient, herself, can do indicate that the ovulatory mucus must consist of several types of secretions. In the spinnbarkeit test the mucus thread is uneven. Thin parts are S mucus and thick parts are L mucus, both are translucent. A beautiful picture showing this has been published by Cohen (1966). The milky parts contain G mucus (sample uncontaminated by vaginal secretion, see fig. 1). If the mucus is applied on a slide, similar observations are easily made. Translucent "hills" are L mucus, translucent "valleys" are S mucus, and milky hills are the G type of cervical secretion (fig. 2). After drying in air, microscopic inspection reveals crystals of

clearly discernible appearance (fig. 3): L mucus has large palm-like crystals, S mucus has thin parallel needles (now ascribed to subtype S2; see fig. 22 for the subtypes S1 and S3), G type has no or irregular crystals. The hill-valley appearance of figure 2 enables a mechanical separation and pooling of various mucus types.

For research purposes, determination of T_1 in proton-NMR is a useful method to distinguish between S, L, and G types of cervical mucus. Figure 4 shows the T_1 distribution in micro-quantities of mucus from separate crypts or secreting units.

- NMR criteria for various types of mucus from single crypts (+21° C) are:
  S mucus, log T_1 is 3.10 or more.
  L mucus, log T_1 is less than 3.10 but more than 2.50.
  G mucus, log T_1 is 2.50 or less.
- If the measurements are performed at +35° C *in vitro* or in NMR tomography, the corresponding limits are approximately:
  S mucus, log T_1 is 3.35 or more (i.e., T_1 is 2,200 msec. or more).
  L mucus, log T_1 is less than 3.35, but more than 2.75 (i.e., T_1 is less than 2,200 msec. but more than 550 msec.).
  G mucus, log T_1 is 2.75 or less (i.e., T_1 is 550 msec. or less).
- The average values of the three kinds or mucus at +21°C are:
  S mucus, log T_1 = 3.25, T_1 = 1,800 msec.
  L mucus, log T_1 = 2.85, T_1 = 700 msec.
  G mucus, log T_1 = 2.35, T_1 = 190 msec.
- At +35 to +37°C the average values are approximately:
  S mucus, log T_1 = 3.50, T_1 = 3,200 msec.
  L mucus, log T_1 = 3.10, T_1 = 1,200 msec.
  G mucus, log T_1 = 2.40, T_1 = 250 msec.

It is important to note that T_1 for S mucus is very close to T_1 for pure water, approximately 3,300 msec., indicating that the fluid for swimming sperm is at least as fluid as ordinary water. It must also be mentioned that we have recent indications that the S mucus alters continuously as it streams downward along the string (see discussion section, p. 16) implying that the S mucus is somewhat more viscid in its lower (distal) part (S3) of the string than in or near

the crypt (S1 mucus). The NMR T_1 values for these varieties of S mucus have not yet been determined. These types are shown in figure 22.

The bulk or flow viscosities involve the mucin molecules and are higher than the NMR viscosities. Values: S mucus, NMR = 1, bulk = 100 cp; L mucus, NMR = 3, bulk = 1,000 cp, and G mucus, NMR = 11, bulk = 10,000 cp. These figures are in reasonable agreement with those of Wolf et al. 1977. Cell countings showed that the cell density in S mucus is rather low (about 1,000/mm^3), somewhat higher in L mucus (about 4,000/mm^3), and high in G mucus (about 15,000/mm^3).

Molecular Composition and Structure in the Three Mucus Types

Electrophoretic studies gave results on the albumin/globulin/mucin relations in agreement with Moghissi' findings (1966).

In special studies, mucus of S, L, or G quality was pooled and dehydrated. The soluble components were removed by diffusion (including albumin and globulins). The remaining mucin was subjected to studies with ESCA, NMR, Rayleigh, or Raman scattering.

The ESCA studies in the sulphur 2 p region of the spectrum showed that the $=SO_4$/-S-S-peak quotient was higher for S mucus than L mucus, indicating a higher amount of sulfate residues in S mucus.

NMR spectra of mucin gelate in D_2O showed obvious differences between S, L, and G mucus (see fig. 11).

The Rayleigh scattering gave information on the molecular Brownian motion. This was most pronounced in S mucus, less in L mucus, and least in G mucus, indicating successively increasing molecular interaction and MW $\approx$ 5-20$\cdot 10^6$D. Raman spectra (figs. 12, 13, and 14) show pronounced water peaks (ω) in S and L mucus, and Raman resonance regions (β) in L and G mucus.

All these studies taken together suggest that the S, L, and G mucin molecules are similar but probably slightly different. These differences might, in turn, help to explain the different forms of molecular aggregations in S, L, and G mucus and their different

water-binding capacities and physiological functions.

Studies on native mucus—quickly separated in S, L, and G components on slides or on single crypt mucus—were performed with NMR proton shift, T_1 and T_2, and Rayleigh and Raman studies. Together these observations give the "models" for mucus architecture shown in figure 5. In this figure, the S mucus is shown to contain mucin molecules in parallel bundles (micelles) with large spaces between them containing a watery fluid. This fluid is the natural medium for swimming sperm. In L mucus, the molecular micelles lie closer together and slow down sperm advance. One of the physiological functions of L mucus is probably to capture morphologically abnormal sperm (fig. 6). G mucus has a much more "split up" and dense molecular network which does not permit sperm advance.

Sperm Penetration

A large number of *slide test* observations confirmed the observations of Bergman 1950 on two types of sperm invasion, "wide front" and "caravans." The wide front invasion is slow and irregular. The caravan invasion is rapid, and the caravans persist for long distances. The caravans start from the "phalanges" described by Moghissi et al. 1964. Cell countings and optical transparency measurements with a TV microdensitometer indicate that the phalanges occur where the S mucus strings meet the semen pool and that the caravans represent invasion in the strings. The wide front invasion occurs in the loafs with L mucus and is irregular and slow. Of course, both loafs and strings and their topography are deformed by squeezing out the mucus for the slide test. Figure 21 gives a schematic illustration of the situation.

Sperm penetration tests on *mucus pillars* with *preserved internal topography* have been most informative. These studies were performed with special microscopic and TV-monitor techniques on "punched out" mucus pillars from the cervical canal (figs. 8 and 9). These studies have revealed the three-dimensional mosaic pattern of S mucus and also indicate a three-peak distribution of frontier sperm. Careful examination of sperm move-

ments within the frontier sperm cluster has shown a kind of cycling behavior which is approximately in accordance with mathematical group theory (fig. 9). This probably implies that the sperm cells communicate with each other. The probable nature of this long-distance intercellular communication has been sought for several years. Recent studies with Raman spectroscopy indicate that sperm emit very-high frequency vibrational or superacoustic quanta (phonons) which are transmitted in water or in micelles and received by other frontier sperm cells; in this way the sperm cells can establish intercellular contacts which facilitate the advance of the most vital spermatozoa. Recent studies (Sjöström 1983) indicate that contractile mobility in the cervix enhances the initial three-peak patterning of the frontier sperm collection which is then probably maintained by intersperm phonon communication. Some of the frontier collection sperm seem to colonize cervical crypts. This residence time in the crypts is characterized by a halftime of 15 hours, thereby prolonging the effective time of a single coitus.

Cyclic Variations

The cyclic variations of the cervical mucus types are shown in figure 7. Post-menstrually, the G mucus dominates.

As the estrogen stimulation on the cervix increases, the biosynthesis of L mucus increases, and, when maximum estrogenic stimulation occurs, the S mucus becomes synthesized and secreted but seldom exceeds more than 30 percent of total volume. The S mucus flows continuously as long brooks (strings) of a watery fluid between the loafs of L mucus. The S mucus is secreted at a high rate and streams rather quickly between the loafs ("like water in a brook streams between pebbles"). Due to the streaming, the mucin molecules become oriented in parallel (fig. 6), interconnect and form micelles, which in turn direct swimming sperm. It seems that the continuous flow of the S mucus is essential for the *in vivo* parallel ordering of molecules to micelles.

Shortly after ovulation, the G mucus is secreted by the lowest crypts in the canal and, by blocking the external os, aids in retain-

ing the sperm which have colonized the cervix after swimming up from the vagina. Figure 16 shows our result on the anatomical distribution of various sections in the cervical region. Cyclic variations on bulk mucus as regards spinnbarkeit, p.c. dry substance, and amount are in accordance with previously published results and are indicated in figure 20.

Figure 10 shows a typical ovulatory cycle. Our own data, based on 34 cycles with twice daily examination, suggest that ovulation occurs $1.9 \pm 0.6(\sigma)$ days before the BBT rise to half of the maximum increase.

Biosynthesis, Secretion, and Water Structure

The biosynthesis of mucus in the cervical mucus probably occurs in a similar way as in other secretory systems in the body. Special enzyme systems may be present in the secretory cells to permit the typical cervical mucus to be biosynthesized.

The parallelism between estrogens and L secretion is evident (Billings & Westmore 1980), but the factors regulating S secretion are not easily understood (see discussion section, p. 16). As previously mentioned, there is NMR evidence that the S, L, and G mucin molecules may be slightly different, even if protein contaminations can not be completely excluded as a possible explanation.

S secretion probably differs from L and G secretion in several important aspects. The S secretion is much more rapid, more variable, and seems more sensitive to neural, emotional, and stress factors. As previously mentioned, it seems essential that the flow or sieving of S secretion is maintained continuously in order to give a stream-orientation of the S mucin molecules.

The S mucin seems to contain higher amounts of sulphate groups, helping to give rise to a special arrangement of water molecules resulting in a very fluid or low viscosity water for swimming sperm (fig. 6). The water structure of S mucus, with high fluidity and pronounced lattice structure, depends on special types of water association to the mucin (fig. 17) and permits long phonon range. The phonons in cervical mucus are recorded in the low-shift part of Raman spectra (fig. 14).

Distribution of Secreting Units in the Cervical Canal

It was found that the G producing units were localized in the lower cervix and the ectopic area. The S-producing units were localized in the upper part of the cervix, and the L-producing areas were distributed more or less along the whole cervical canal.

The so-called "iso-units" (non-cyclic glands) were found mainly in the transformation zone. Figure 16 shows this distribution.

The Intracervical Mucus Architecture

Figure 15 shows a picture or "model" of the distribution of various mucus types in the cervical canal (*in vivo*) at the time of ovulation. It is a "dynamic mosaic" pattern of mucus. The L mucus units (loafs) lay like pebbles in a brook, and the watery S mucus is running between them. The loaf system is also successively but slowly replaced by the release of new units from the L-secretory areas. As mentioned, the string system of S-mucus is, therefore, also variable; new interconnections between strings re-form, others are broken. The whole system is dynamic, but its main structure remains for several days: Thin sperm-conducting strings of liquid mucus are imbedded in a system of soft mucin "pebbles."

The Isthmus

Very few samples could be obtained from the isthmus region. They contained no mucus, only a grey-yellowish serous liquid.

Some samples were used for micro NMR T_1 measurements. Some were frozen, dried and pooled, and redissolved in D_2O for diffusion purification of the possible sperm-activating factor and for use in high-resolution NMR spectra.

The Vagina

The vaginal samples were handled in different ways. Some were directly dried for ESR studies. Others were separated into cells and supernatant before ESR. Only supernatant obtained from lower vagina and paraurethral pockets showed the typical manganese ESR spectrum with six lines (fig. 19).

Other samples were used for NMR (fig. 18) and dry-substance measurements. The vaginal secretion may have the function to set up a pH gradient in the spermatic pool, thereby orienting or "focusing" the sperm on the external os and the cervical mucus plug. The manganese in the vaginal fluid (fig. 19) may have a similar function mediated by manganese-zinc interaction. Proton-NMR of vaginal fluid (fig. 18) shows a barrier between intra- and extracellular water at midcycle, something that may aid in giving vaginal fluid its biological function in fertility.

When extracervical mucus was found in the vagina, pieces of this mucus were removed for investigation. Similar methods as for intracervical mucus were utilized. Usually the T_1 values were similar to the intracervical bulk mucus values. The contamination with vaginal contents, however, made the interpretation of these values difficult.

In 113 women, the location of mucus in the cervix and vagina was noted in the periovulatory period and compared with each woman's own experience or feeling of the mucus location. The results are shown in table III. The agreement between observations and the patient's own report is sufficient as regards the location in the lower genital tract. It is remarkable, however, that some patients said they had a feeling deep in the body when the mucus was localized mainly in the cervical canal. This sensation had a component of pressure to urinate but also a slight projection to the sacral region. Figure 23 gives some information on the circulation of material in the vaginal lumen (Odeblad 1964) and the locations of mucus found in this study.

Discussion

We are now in position to discuss the findings on the cervix and vagina and the interaction between cervical and vaginal secretions with respect to normal sperm transport, fertility problems, and NFP. We will first look at the anatomical distribution of secretory units in the cervix, then at the hormonal and neural regulation of these units and the resultant mosaic distribution of mucus in the cervical canal as well as its dynamics. Also cervical