pathology will be considered. We will then look at the vagina and at the sperm transport in the combined cervico-vaginal system. Finally we will discuss the cervico-vaginal system and its role for NFP.

A. Anatomical Distribution of Different Secretory Units

The distribution of secretory units in the cervix is essential for the fertility mechanisms to operate optimally. Normally there seems to be a preponderance of S secreting units in the upper half, while L producing units are present in the whole cervical canal but dominate in the canal's lower half (fig. 16). The G producing units are located around the external os. During the days after ovulation, the G secreting units become activated, and thick, sticky G secretion effectively locks the external os which also decreases in diameter. In this way the external os becomes more or less closed, and the intracervical sperm has only one way to swim, upwards.

One question which has often been discussed is whether there are separate units secreting the S and L types of mucus. To date this question can not be answered with certainty, but present data indicate that there may be separate units for these two functions. On the other hand, recent studies indicate that the G secretions produced during the postmenstrual and the postovulatory periods are, in fact, somewhat different. It seems, therefore, that progesterone is capable of slightly modulating the function of the G-producing units.

In the isthmus region, there is another type of gland, differing histologically and functionally from the cervical structures. There is evidence that the isthmus secretion is capable of stimulating sperm motility, and a low-molecular factor, tentatively called axreveillin (Odeblad 1975), may be responsible for this effect. Axreveillin has been partially purified. It seems to be a labile compound, something that has hitherto prevented detailed studies. There are, however, indications that it contains a catecholamine-like residue, a few amino acids, and a monosaccharide unit with a MW of about 3,000.

A special condition seen normally, but also after longstanding cervical infection, is the occurrence of so-called iso-units in the cervix. These secretory units do produce their mucus independently of hormonal stimulus, while the normal cyclo-units are dependent on the cycle phase for their activity. For example, the iso-L-units always produce L-secretion even during the post-ovulatory phase, or even in early pregnancy, and the iso-G-units are usually located on the portio, in the transformation zone, covered by regenerate squamous epithelium due to metaplasia. The significance of these glands is unknown.

B. Cyclic Variations and Secreting Units

The cyclic variations shown in figure 7 for the various mucus types require some comments. It is noteworthy that the L secretion appears several days before the S secretion begins. As pointed out by many authors (e.g., by Billings & Westmore 1980 and authors referred to by them), there is a striking parallelism between the concentration of estrogen levels in plasma and the appearance of the first mucus symptom as well as the cyclic curve of L mucus production.

As is evident from figure 20, there is also a parallelism between the peak symptom and the S mucus. The period of S mucus secretion is about 3 days. This is in agreement with Bergman's results (1950) that the mucus is sperm receptive 2-4 days before the BBT shift. The peak symptom is considered to be the time of maximum fertility.

The factor(s) regulating S secretion is (are) not known. One possible mechanism is that estrogens start the synthesis of a membrane or cytoplasmic receptor, necessary for S formation, and some days are required to develop this receptor. The increase of the E1/E2 quotient around ovulation (Landgren 1977) might induce the S-secretion. A third possibility is that the FSH or LH itself could start the S biosynthesis. Also a neuroreflex from a distended follicle is possible.

The S production seems to be sensitive to stimulation of the vegetative nervous system; both inhibition and increase of secretion seem to occur, depending on emotional and autonomic factors. We have, for example, seen that female students under the stress of an exam can produce excessive amounts of S-like cervical secretion, and there may be some similarity to the secretory activity of the sweat glands or lacrimal gland ("the weeping cervix"). The G mucus seems to be stimulated by gestagenic hormones, both natural and certain synthetic gestagens.

It is presently difficult to determine the exact time relationships for the cervical events before and around ovulation. Figure 20 may give some information. The LH surge is, in itself, not precisely defined. Several partial peaks occur within one day or more (Landgren 1977). According to Burger 1981, ovulation occurs about 17 hours ($\approx$ 0.75 days) after the LH surge, with a wide range of time. From the data published by Brown et al. 1982 and Billings 1981, we can infer that the BBT rise occurs, on the average, 2.25 days after the LH peak, and the data of Moghissi et al. 1972 give about 2.75 days for the same time interval. We therefore consider 2.5 days as an acceptable value of the LH - BBT interval. This means that the morphological ovulation would occur approximately 1.75 days before the BBT rise. Our own data give 1.9 $\pm$ 0.6 days based on bimanual ovarian examination. This figure also depends on our definition of BBT rise, and Matthew et al. 1980 have even suggested two types of BBT rise.

C. *The Mosaic Mucus Pattern in the Cervical Canal*

Many years of studies have resulted in different proposals for the distribution of the mucus types S, L, and G inside the cervical canal of a woman. Figure 15 shows the most probable situation during the ovulatory period. Each S-type secretory unit produces the S mucus continuously. It flows downwards between loafs of L mucus. As a consequence of this flow, the mucin molecules line up in parallel and intermolecular interaction tends to aggregate the mucin molecules into long filaments called micelles, also ordered in parallel. The loafs are secreted in a somewhat different manner. *Each L producing area or unit becomes filled by gelatinous secretion which is then suddenly released as a* *whole*. This type of release has been watched in the colposcope several times. Then the filling up of the L type cleft or crypt begins again until the next emptying event occurs, 1-6 hours later.

Totally there may be about 400 strings and some 2,000 loafs in the cervical canal at midcycle. The strings are 50-100 μm in cross section, the area of cross section being 0.05 x 0.1 mm and irregular, star-shaped, and varied. Depending on the location, the strings are 15-30 mm long. Each loaf is ellipsoid, about 0.5 x 1 x 3 mm in size. The system is shown in figure 15.

The most important function of the SL system is indicated in figure 6. High quality sperm will probably pass rapidly upwards inside the string. Low quality sperm, with irregular motion or angulated head-tail junctions tend to become captured by a loaf and "imprisoned." Later on, these sperm become expelled by bulk flow of secretion. Accordingly, the SL system acts as a biological filter, probably favoring the advance of high quality sperm.

As previously mentioned, the flow of S secretion seems to be essential for rapid sperm penetration by orienting the mucin molecules in parallel. There is also evidence that the S mucus "steals" molecules from the L mucus, with the effect that the S mucus changes a little on the way from the crypt to the external os. In fact, we can identify three stages of S mucus due to this process; we call them S1, S2, and S3 from crypt to external os. The "original" S1 mucus contains little mucin, S2 more, and S3 still more. The three variants of S mucus have different crystallization patterns (Höglund, Perenyi, & Odeblad 1983). Figure 22 depicts the situation schematically.

Preliminary observations indicate that, if the flow of S secretion is rapid, the admixture of L-type mucin molecules is less, and the S mucus retains its S1 character more or less within the whole string. If the flow is slow, the S2 and S3 character becomes more evident. If the flow ceases completely, as it does in a sample removed from the cervix, then a more or less complex mixing of S and L secretions occur within a couple of hours, and the S and L mucus cease to exist as distinguishable entities. If we want to study the internal mosaic structure of cervical mucus, we must therefore (a) remove it very gently, so that mechanical mixing does not occur and (b) investigate the sample immediately so that mixing by thermal diffusion of molecules, including mucin molecules, does not occur.

D. Pathology

The mechanisms described may be sensitive to pathological processes. Congenital and constitutional factors may influence the relative contribution of S and L secreting structures so that sub-optimal proportions of the mucus types are secreted, something that may reduce fertility. Also, emotional or organic disturbances of the neural control of secretion may occur. As mentioned, it has been recognized several times in our studies that acute emotional stress exaggerates the flow of S secretion so that the cervix may be completely filled with a thin liquid, incapable of orienting the sperm advance in an upward direction.

Another important group of suboptimal conditions results from hormonal disturbances. Low estrogen levels have long been known to be associated with inadequate sperm penetration. This condition can be favorably improved by treatment with estrogens or gonadotropic hormones, depending on the clinical findings.

Probably the most common cause of cervical dysfunction is cervicitis. Cervical infections can be acute, subacute, or chronic. The localization of the inflammatory condition can be generalized in the whole cervical canal or restricted to a few clefts or crypts. All these conditions can influence sperm penetration in several ways. Even the presence of only a few large cystic glands may impair the function of the cervix as a whole, if the lumen or neighbor crypts are compressed or they act as foci for acute exacerbations. It is common to subject patients with cervicitis to antibiotic treatment, but a carefully undertaken microsurgical treatment is probably more adequate for long-time healing (Rudolfsson-Åsberg & Odeblad 1971a).

E. The Vagina

The role of the vagina is often overlooked and, to a large extent, less known than the cervix. It is generally believed that the pH gradient between the vagina and cervix might have a "chemotactic" effect, orienting sperm swimming upwards. We have studied five other problems:

1. The physical state and movement of water in the vaginal lumen.
2. The physical state and movement of cervical mucus in the vagina.
3. The physical state of water and proteins associated with vaginal cells during the cycle.
4. The presence and role of manganese in the vagina.
5. The mucus membrane topography in the lower vagina.

There is a natural convection of material from the cervix downwards (Odeblad 1964). This may be due to a natural reabsorption of water from the lower vagina. If mucus is transported quickly in this way without disintegration, it can be felt and observed in the lower vagina and vulva. If the mucus transport is slower and disintegrates more quickly, mucus will not be felt at the vulva level.

Apparently several factors play a role in the possibility of observing mucus at the vulva and lower vagina:

1. The cervical secretory rate.
2. The quality of mucus (S, L, or G).
3. The speed of intervaginal mucus transport downward.
4. The speed of intervaginal mucus disintegration.
5. The vaginal contents, themselves (normal or infected, hormonal stimulus).
6. The reabsorption of aqueous material in the lower vagina, probably related to the area available for resorption.

It is not yet possible to understand all the steps in the mechanisms relating to mucus observation.

The speed of intravaginal transport is probably dependent on muscular activity in general and specifically in the levator muscles as well as positions and movements of neighboring organs such as the bladder and rectum. As regards manganese, it may play a role as activator or inhibitor of mucus disintegration, and it may also interact with zinc in the semen, making sperm cells more apt to fulfill their transportation or biological purpose. Further research is needed on this point.

We have found that the resorptive capacity of the lower vagina is largely dependent on two factors: (1) the depth of the para-urethral pockets of Shaw and (2) the surface enlargement of the lower vagina and the pockets by the papillary structure of the mucus membrane.

F. Sperm Propagation

Studies on sperm swimming in "punched out" mucus pillars have shown the specific sperm distribution pattern mentioned above. The sperm move in S mucus, and a three-peak distribution of the pioneering cells becomes established. Detailed studies have revealed that each sperm alternates between the three distributions. If it leaves the last one, it permanently remains in the tail region.

A mathematical analysis of these motion phenomena has been performed, and all evidence conforms with the assumption that a profound principle of nature, called group theory, is acting. There are several mathematical groups known, and the type of group which seems to regulate sperm behavior is a so-called Lorentz group, which is known to play an important role in many physical phenomena. Sperm advancing along a string may proceed directly to a crypt. These crypts become colonized by sperm within a short time, 15-60 minutes post coitus. Some strings, however, have become fractured and joined with other stream-lines so that rapid ways lead directly to the internal os and uterine cavity. In this way rapid advance of sperm to the uterine cavity can occur, probably assisted by uterine contractions (Ingelman-Sundberg & Odeblad 1980).

As discussed earlier, many observations of the "punched-out" mucus plugs (with preserved structures) indicate that at least some kinds of malformed sperm have a tendency to accumulate in the L mucus lobes. These units of mucus have irregular micellar orientation, and the sperm entering a loaf of L mucus swims slowly in irregular paths and has little probability of escaping back to a string. In this way, most sperm captured in the loafs are "imprisoned." The whole loaf system moves slowly downward in the cervix because new loafs become successively formed and expelled in the cervical lumen. In this way the imprisoned sperm are carried downward in the canal and finally enter the vaginal lumen where they are later transported out of the body.

We must again discuss a little more about the slide test, the capillary test, and the punched-out pillar test. Davajan and Nakamura 1972 discussed reliability and errors of the slide test. They showed that artificial stretching of ovulatory mucus aligned the mucus molecules, something that artificially directed the swimming sperm. After drying, a so-called "channeling" occurred. This was also pointed out by Davajan, Nakamura, and Mishell 1972. Some authors have later used such channeling studies as an index for the mucus quality. We would like to point out that this channeling has very little to do with the natural channels occurring in the intact mucus *in vivo* or with the nearly intact mucus we can study on the punched-out specimen. Such specimens have nearly intact internal mucus structure, in which sperm follow the naturally occurring channels in the S mucus, responsible for the *in vivo* sperm transport. In the capillary test, the situation is also artificial. When the cervical mucus is forced into a capillary, the natural architecture of loafs and strings is completely destroyed. The loafs are compressed and elongated and the mucus molecules and filaments become aligned more in parallel. This facilitates the sperm propagation, but does not at all reflect the *in vivo* situation. The slide test also destroys most of the architecture of the mucus, but has some advantages: (1) It enables us to see the loaf-string structure at the border of the sperm pool by observing phalanges (fig. 21), and (2) the optical conditions for observation are better than in the capillary test.

In punched-out specimens the whole string-loaf system is more or less preserved, and the strings can be followed under the microscope. This is not, however, as simple as it sounds. Because the tube is circular, it must be optically embedded in a water layer between a slide and a cover glass, and we have to use low magnification to observe the considerable depth (5 mm) of the specimen. It requires a lot of training to remove and observe the samples, and, to date, only perfectly cylindrical cervical anatomy has permitted such samples to be removed for studies without distortion. Thin-wall glass tubes with an ellipsoid cross section may, in the future, widen the applicability of this test which can be used either for postcoital testing or for *in vitro* studies of sperm invasion and penetration.

G. Remarks on the Detection of Ovulation

It has become customary in modern medicine to utilize more and more complicated methods for diagnosis, and to rely more and more on different tests rather than on the patient's self-observations and the doctor's own examination. This trend applies also to the detection of ovulation. The simple principle used by Dickinson and Hartman to manually examine when ovulation occurs is almost completely neglected nowadays. One of the latest technical aids in this field is ultrasound. We believe one must be rather careful when using ultrasound. It has not been definitely proven harmless; instead some data indicate that diagnostic doses of ultrasound may cause alteration in DNA molecules (Liebeskind et al. 1982, Ciatti et al. 1982). Until the complete freedom from any risk is definitely proven, we must be very restrictive in the use of ultrasound, especially in irradiating the ovarian tissues with its oocytes which will in time mature and possibly become fertilized.

H. General Remarks Pertinent to NFP

The qualitative and quantitative changes in cervical mucus in the ovarian cycle and their relation to NFP methods have been discussed by many authors, e.g., Billings et al. 1972, Flynn & Lynch 1976, Casey 1977, Vollman 1977, Hilgers et al. 1978, Billings & Westmore 1980, Billings 1981, Burger 1981, Cortesi et al. 1981, Brown et al. 1982. On the basis of the results and discussion presented in the present paper, we hope that a firmer basis will be established for the practical application of NFP based on the woman's recognition of the varying degrees of fertility throughout the menstrual cycle. We have tried to find relations between our own findings and the findings of other researchers in such a way that the basic biophysical properties of the cervical and vaginal secretory, transport, and other biological functions can be interpreted in practical terms.

First of all, we would like to draw attention to the existence of three different types of cervical secretions, now called S, L, and G. The post-menstrual G secretion is superceded by the L secretion when the circulating estrogens rise, and the woman experiences the first mucus symptom, which increases in intensity. This soft, mucinous secretion turns into a slippery, watery secretion a few days before ovulation when the S secretion occurs from the cervix. We do not exactly understand how the S secretion is regulated. Shortly after ovulation, both L and S secretions disappear and the postovulatory-premenstrual G secretion occurs, apparently as a result of progesterone action on the cervix. A certain balance between S and L secretion is probably necessary for optimum fertility, and inside the cervical canal the topographical relation of S mucus in long brooks or strings between "pebbles" or loafs of L mucus is, in all probability, essential for high sperm transmission efficiency. The optimum of S secretion probably coincides with the peak symptom recognized by the woman, and this disappears with the shift to G mucus. The intravaginal downward transport in the vagina is probably very important but also, in part, less known than the role of the elements manganese (present in the vagina) and zinc (present in the semen). Much work still has to be done on these and other questions, but we hope that our biophysical studies so far have provided a better understanding of the physiological bases for NFP.

Summary

To summarize our new aspects on cervical mucus and NFP, we want to make the following points:

1. The ovulatory mucus is a mosaic made up of mucus strings and mucus loafs. The strings contain a fluid gel, S mucus, and the loafs a more viscid gel, L mucus. The S mucus is very thin and flows rapidly between the loafs of L mucus. The strings are about 100 μm in diameter and 2-3 cm long. The loafs are ellipsoid and 0.3 x 1 x 3 mm in size. Near the external os, there are some

units of still more viscid G mucus.

2. In approximate terms, the S material is 30 percent and the L material 70 percent of midcycle mucus. There is, however, a day-to-day percentage variation due to the continuous secretion and outflow of cervical mucus. The mosaic structure of the endocervical contents is not static but dynamic.

3. S and L mucus are secreted from different secreting areas or units (clefts, crypts, glands) in the cervical canal. The upper part of the cervix tends to produce most of the S mucus (strings).

4. The S mucus flows quickly between L loafs. This flow orients the mucin molecules, which thus tend to form long thin aggregates, called micelles, separated by a waterlike medium, permitting very rapid sperm advance.

5. The "anatomy" of the mucus plug mosaic is such that some sperm can directly swim to the neighborhood of the uterine cavity. Most sperm, however, are conveyed to the corresponding S secreting crypts, in which they seem to hibernate and form a sperm reservoir with a half-time of about 15 hours.

6. The ellipsoid units of L mucus seem to act as a mechanical support for S mucus, sieving between the loafs. They also act as a trapping mechanism for sperm which are presumably less suitable for fertilization. The trapped sperm are probably later expelled with the bulk cervical mucus.

7. The advance of the high-quality sperm in a string seems to be a highly ordered process, in formal agreement with mathematical group theory (a discrete variety of a Lorenz-like group). This indicates that some kind of intercommunication between the sperm cells exists, phonons being the most probable candidates of intercellular messages.

8. In the menstrual cycle, the first mucus symptom seems to occur when the soft L mucus first appears. The peak symptom of mucus discharge coincides with the maximum of the slippery S mucus secretion. When S mucus disappears, the dry days begin.