The Discovery of Different Types of Cervical Mucus

and

the Billings Ovulation Method

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Abstract

An introduction to and some new anatomical and physiological aspects of the cervix and vagina are presented and also an explanation of the biosynthesis and molecular structure of mucus.

The history of my discoveries of the different types of cervical mucus is given. In considering my microbiological investigations I suspected the existence of different types of crypts and cervical mucus and in 1959 1 proved the existence of these different types.

The method of examining viscosity by nuclear magnetic resonance was applied to microsamples of mucus extracted

outside of several crypts. Preliminary studies in 1966 proved the existence of two types and in 1977 the three types G, L, and S mucus were described. Sperm cells were transported in S mucus, L mucus attracted malformed sperm and G mucus formed a plug in the cervical canal in the infertile phases.

In 1990 a new mucus, P mucus, was characterized. A mucolytic enzyme, probably emanating from the isthmus of the cervix, was associated with this mucus and facilitated the upward movement of sperm cells. At the end of 1993 another mucus, F mucus, was identified. This mucus is probably produced by fetal cells remaining in the wall of the neck of the uterus.

Variations in these different types of mucus throughout life and during the course of a cycle, and their importance for the Billings Ovulation Method, are presented and discussed.

Introduction

I am very pleased to present my research on the different types of secretion from the neck of the uterus [cervix] and their relationship with the Billings Ovulation Method (Billings et al. 1972; Billings 1983; Billings and Westmore 1992).

The principal indication of fertile days in a menstrual cycle is the appearance and the sensation of a wet substance [mucus] emanating from the epithelial membrane of the cervix. 'Ibis sign precedes ovulation and depends upon the growth in the ovary of a follicle which produces oestrogens and which is usually succeeded by the rupture and the release of an ovum (ovulation). The last day on which this substance with fertile characteristics is observed is called the Peak day. This day is also the day of ovulation in 80% of cycles. Ovulation occurs on the preceding day in about 10% of cycles and in about 10% of cycles on the day following the Peak.

Besides the cervical secretion there is also a maturation and cellular sloughing (of superficial squamous cells) of the vagina. There are also some contributions emanating from the isthmus, the endometrium and the tubes. It has also been demonstrated that peritoneal fluid and, during ovulation, follicular fluid contribute to the cervical flow.

Usually, in the infertile phases, vaginal fluids are reabsorbed by the pockets of Shaw (vaginal recesses), situated in the lower part of the vagina (Odeblad 1964), and the element manganese plays a role in this process (Rudolfsson and Odeblad 1971). The preovulatory epithelial proliferation lessens the reabsorption and the flow outside the vagina increases and, as a consequence, facilitates the wet, slippery sensation in the fertile phase.

Since the beginning of the 20th century it has been known that the ovarian cycle causes variations in the secretions of the mucous membrane of the cervix. References to that work are found in Billings (1983). &t that time it was generally supposed that the secretion of the glands (or crypts) of the cervix changed in a manner more to synchronize with the course of the cycle. I wish to report that the directives or rules elaborated by Drs John and Evelyn Billings have not been influenced by my research. Those rules are always valid.

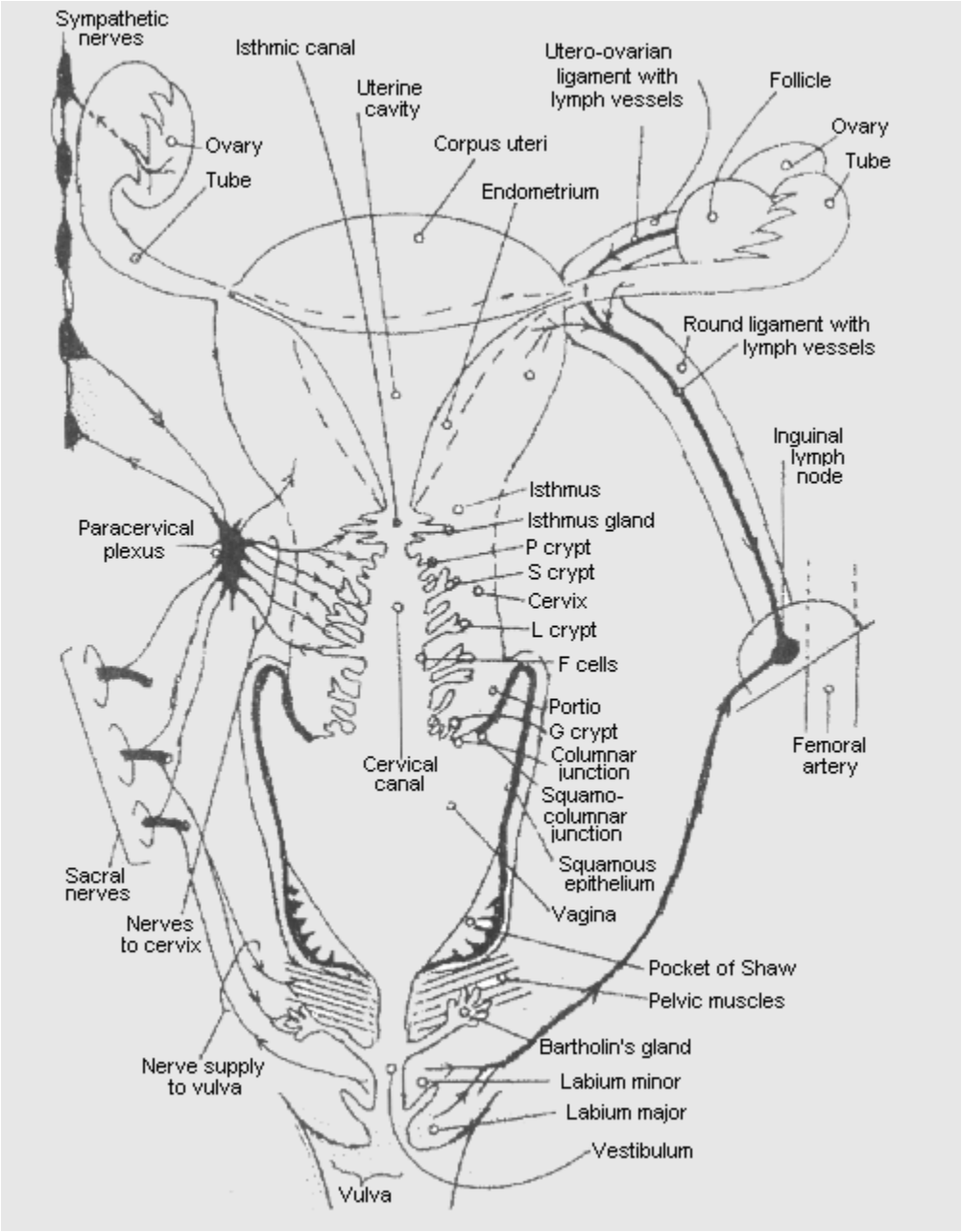


Figure 1. Important anatomical structures of the female genital system. nerve supply is indicated on the left and the lymph drainage on the right.

Anatomy and Physiology

I know that you are all very familiar with the anatomy and physiology of the female genital tract but I draw your attention to some new facts (cf. Figures 1 and 14). The isthmus is a part of the uterus, localized between the body and the cervix and with a length of only 5-7 mm. Its glands produce a fluid, secretion Z, which probably contains several enzymes.

The cervix has four different types of crypts, denoted G, L, S and P, which produce the four types of mucus: G, L, S and P mucus. Also, between the openings to the crypts there are some cells, F cells, which are not differentiated. These cells produce F mucus which probably does not have any physiological function but the G, L, S and P types have their specific functions in the reproductive process and have importance for the symptoms of fertility and infertility.

There are two pockets of Shaw in the distal part of the vagina (vaginal recesses-Krantz 1959) which are able to absorb water and matter of low molecular weight. Manganese (Mn) plays a part in this process.

The function of the nerves of the cervix are not well known. However, we know that the neurotransmitter noradrenaline has a stimulating effect on the secretion of S mucus.

My research has shown that there are some crypts which perhaps are localized in such a way that two different types of crypts have a common orifice (Figure 1). Another variation of the anatomy and physiology is a fusion of two neighbouring crypts. The crypts are joined to form one crypt that has two openings. This fusion can happen during pregnancy.

The lymphatics of the genital tract, especially one of the lymph nodes in each groin, have a special importance as an indicator of ovulation (Figure 1). This depends in part on the connection between the ovary and the groin along a fibrous string called the gubemaculum during the early embryonic development (Figure 13a). These problems will be considered in a forthcoming paper.

What is Mucus?

Cervical mucus is produced by the biosynthetic activity of the secretory cells of the cervix and contains three important components: (1) mucus molecules; (2) water, (3) chemical and biochemical compounds (sodium chloride, protein chains, enzymes, etc.).

Mucus molecules have two important properties: (a) they are able to join together to make polymers or an extended three-dimensional network (i.e. a gel); (b) since they are glycoproteins their properties can vary widely. Thus different types of mucus are produced, for example G, L, S, P and F mucus, which form different networks or gels. Also, other substances--ions, protein chains, and enzymes--are able to modify the interaction of the mucus molecules and, as a consequence, their biophysical properties. The molecular weight of mucus is about 70,000 daltons, and it is believed to be several million daltons for gels. A general structure of a gel is given in Figure 2.

Because of these structures mucus is not a normal but an abnormal (i.e. non-Newtonian) fluid (Löfdahl and Odeblad 1980) and its viscosity is not able to be measured using liquid-flow techniques. It is therefore necessary to use other methods, preferably nuclear magnetic resonance (NMR) techniques which do not involve flow, but make use of thermal movements of molecules in the fluid (James 1975).

As well as the glycoproteins which are secreted, the cells produce membranous glycoproteins which are bound to the cell membrane. These glycoproteins enable immunological identification of the cells. A similar product is the substance located in the basal membrane which is probably secreted by the cells of the mucous membrane, as are also the cell adhesion molecules.

There are three groups of cells in the mucous membrane of the cervix: (1) cylindrical (columnar) secretory cells (the majority); (2) cylindrical (columnar) ciliated cells; (3) "reserve" cells. The origin of the secretory cells is known but the mode of development of the two other groups of cells has not yet been decided. The cells of the mucous membrane are slowly detached and are displaced with the mucus. New cells are formed to replace them.

The biosynthesis of mucus is a complicated process. The epithelial cells are stimulated by oestrogens (S-, L- or P-cells) or by progesterone (G-cells). The hormone is bound to a receptor in the cytoplasm, and is then transported to the cell nucleus. The receptor + hormone complex then activates certain parts of the genetic material (DNA) which is transcribed to another type of genetic material (RNA) which, in turn, carries the genetic message to the place in the cell where the amino acids are arranged in the correct sequence to form the protein core of the molecule. Carbohydrate molecules are then attached by enzymes onto the protein core. The instruction to the cell how this should be done is probably different in cells of type S, L, P and G by information laid down in cells already during their embryonic development (see below).

The response of the cells to oestrogen or progesterone stimuli is comparatively slow (from one to several hours). The S and P cells also seem to have access to a much faster response mechanism, the stimulation by noradrenaline acting on a beta receptor localized in a cell membrane. This response occurs probably within a few minutes and may be responsible for the "instantaneous" discharge that some women experience on acute stress, e.g. in "stage fright" or a sudden emotional upset.

After the preceding presentation of some basic information, I shall now turn to the discovery of the various types of mucus.

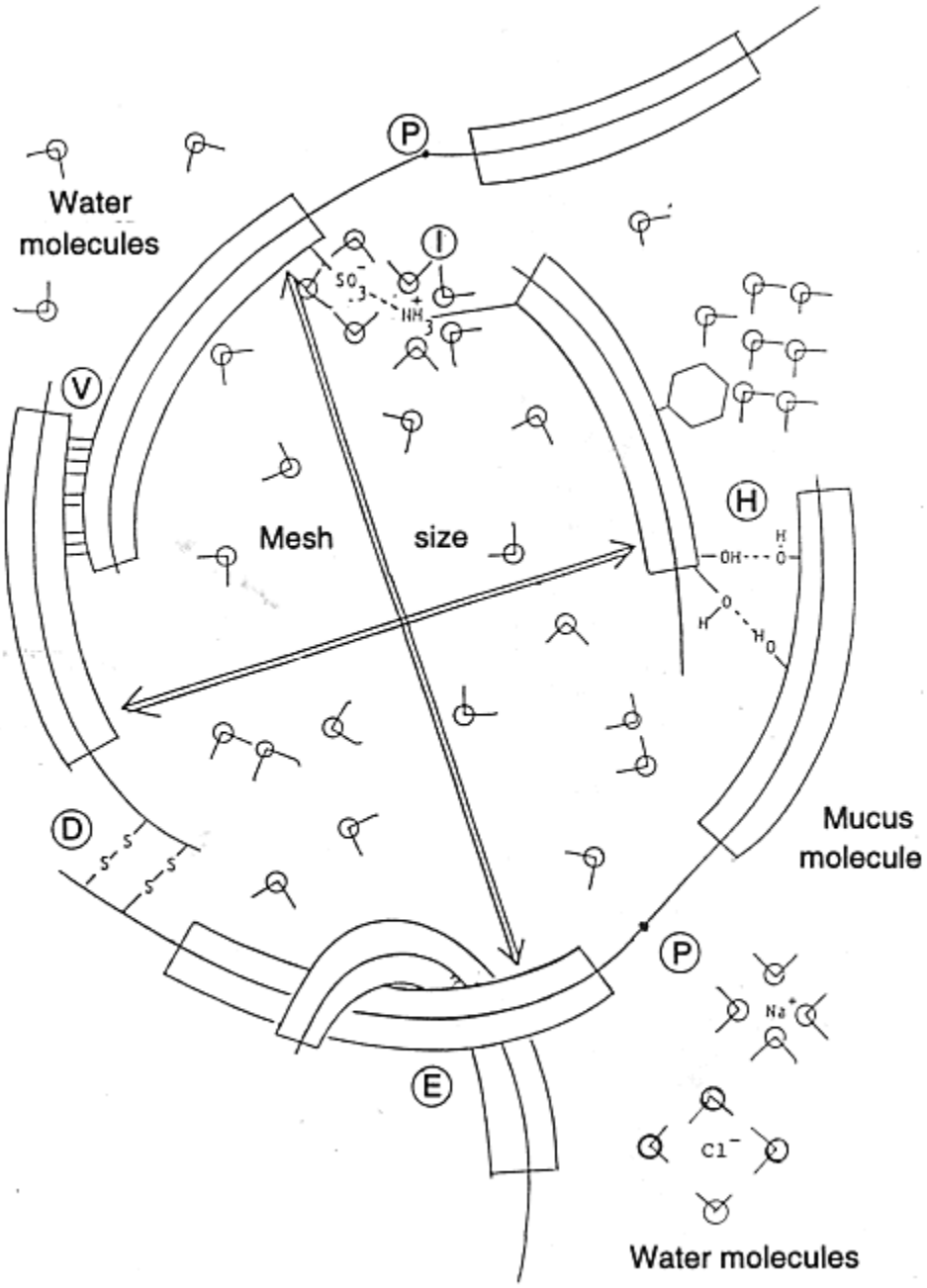


Figure 2. Molecular structure of mucus. I, ionic bond;P, peptide bond;H, hydrogen bonding;D, disulfide linkage; V, van der Waals bonding; E, entanglement of mucus molecules.

The Commencement of my Research

My research on the cervix began in 1949 during the course of my microbiological studies, which were concerned with mycoplasms in the genital tract of healthy, and sick women. I was responsible for the gynaecological examinations and the collection of microbiological specimens. This research was published in 1951 and 1952 (cf. M,len and Odeblad 1951, 1952). We made some interesting observations in the healthy women examined during the pre-ovulatory and post-ovulatory phases:

(1) Mycoplasms were cultivated several times in 5 out of 32 non-pregnant married women but in not one of 13 virgin women or in 11 additional virgin women studied by Frisk et al. (1952) (Chi-squared test, $P< 0.05$). This result was in

accordance with a sexual transmission of mycoplasms, as proposed by Dienes et al. (1948).

(2) In the healthy married women 6 out of 17 cultures were positive in the first part but none were positive in the last part of the cycle (Chi-squared test, $P < 0.01$), an indication that post-ovulatory mucus was able to exercise an antimicrobiological effect (Barton and Wiesner 1945; Pommerenke 1946). 1 shall return to this problem later.

During the collection of samples I witnessed the characteristic variations in mucus in the course of the cycle. I also happened to read three important papers. The first was a review by Esselbom (1947) on cyclical variations in cervical secretions, the second a paper by Rydberg (1948) on crystallization of cervical mucus and the third a paper by Bloembergen et al. (1948) on the new NMR method for measuring viscosity.

The Existence of Different Types of Crypts and of Mucus

I have already mentioned the cyclical variation of the cultures of the mycoplasms. We observed another interesting situation: in three married, healthy, non-pregnant women mycoplasms were recovered in the same cyclical manner despite the fact that it was not possible that these women had become re-infected. If all crypts produced an antimicrobial mucus in the post-ovulatory phases of the cycle all the mycoplasms would be killed and hence not able to be recovered. Then, in 1952, 1 suspected that the mycoplasms could survive in secretory inactive crypts after ovulation.

To investigate the problems associated with cervical crypts I studied biophysics at the University of California, specializing in NMR and radioactivation techniques. After my return to Sweden I commenced in 1954 to apply these methods to the study of the secretions of cervical crypts. In what follows, only the application of NMR is described. Radioactivation studies have supported the conclusions obtained with NMR.

In principle, two approaches were possible:

(1) Investigations on intracanalicular mucus - macrosamples (Figure 3) which were possibly a mixture of two or several types (Odeblad and Bryhn 1957).

(2) Investigations on mucus obtained from within crypts - microsamples (Figure 4) (Odeblad 1966b).

The investigations of the macrosamples were comparatively easy but those on the niicrosamples required new, extremely precise equipment which required several years to develop. During these years I found in the medical literature some support for the hypothesis for the existence of different crypts. Observations by Roland (1958) on crystallization and by Montgomery (1959), on the composition of mucus stimulated my research and in 1959 I presented for the first time the results of microscopical examinations which showed that cervical mucus was composed of several different types which are produced by different crypts (Odeblad 1959).

In 1966 (Odeblad 1966b) I succeeded in proving, by examination of microsamples, the existence of crypts ("glands") which responded differently to the same hormonal stimulation. Among 70 crypts studied NMR recordings and slide samples indicated that 38 crypts contained only one type of mucus with low or high viscosity, and 11 crypts probably contained a mixture of mucus with both high and low viscosity. 21 microsamples were contaminated during the extraction procedure. The existence of crypts which contained two types of mucus was later proved and published by my collaborator (Rudolfsson 1971). Probably these crypts have two branches with a common opening (Figure 1), the branches having different secretory functions.

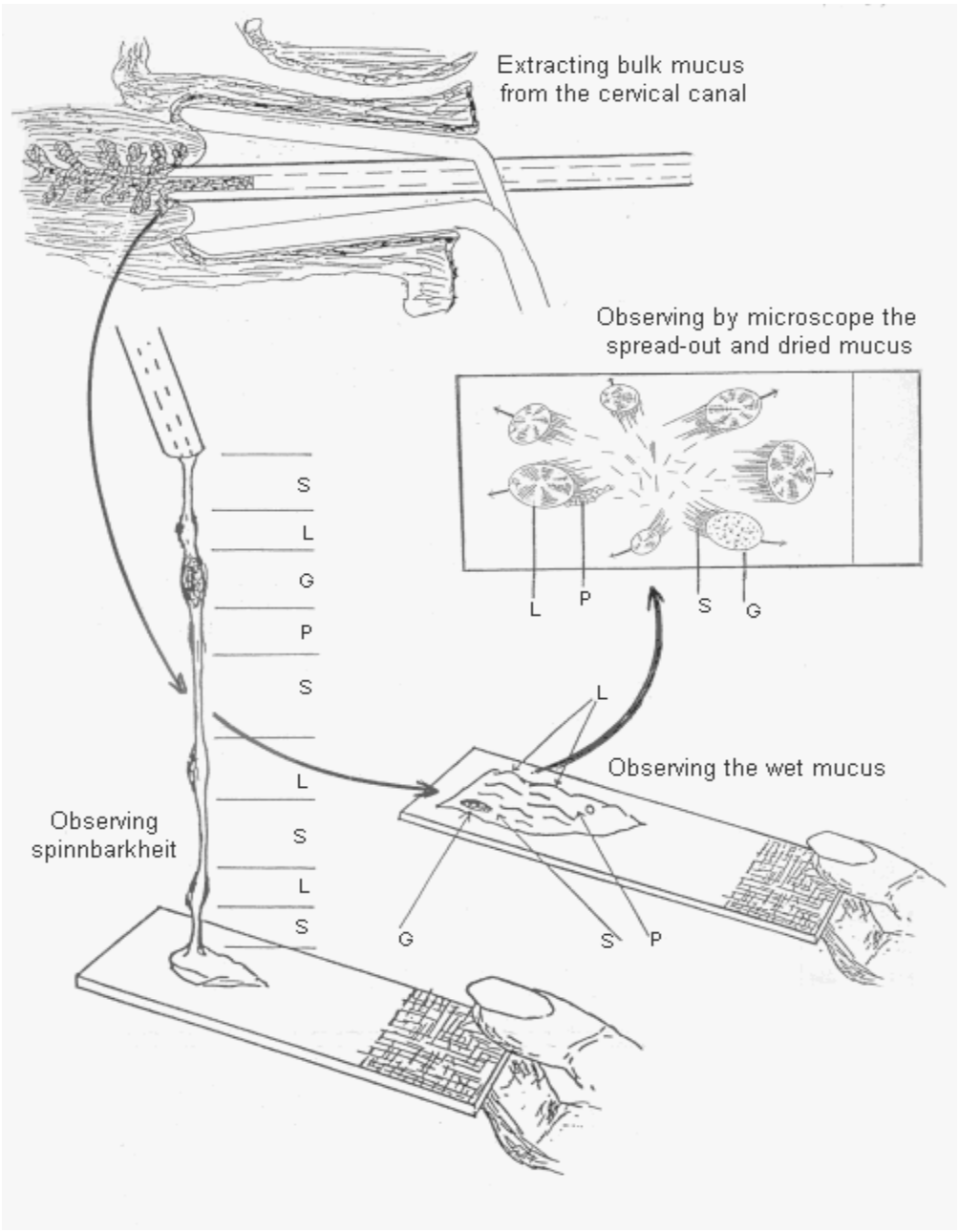


Figure 3. Microsample of mucus in the cervical canal.

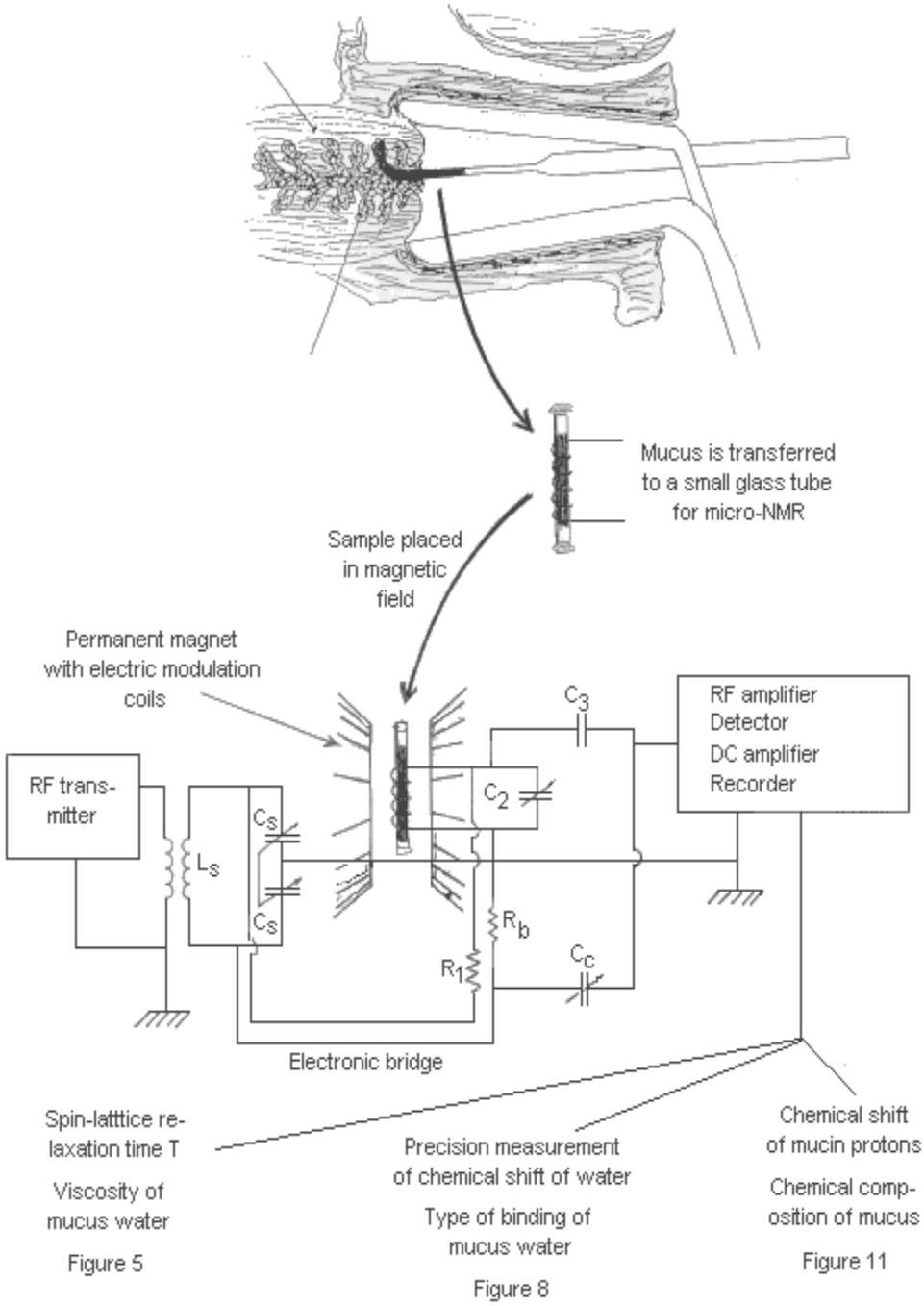


Figure 4. Microsample of mucus in a cervical crypt.

Identification and Description of G, L and S Mucus

At the end of 1968 1 had identified and characterized two types of mucus, one with a high viscosity (G) the other with a low viscosity (E). E mucus was stimulated by oestrogens and G mucus by progesterone, G mucus being produced in G crypts, E mucus in E crypts. Also we isolated the liquid phase (aqueous, in the lattice structure) of mucus. This phase was named secretion B since it resembled blood serum (Odeblad and Rosenberg 1968). The B component is temporarily secreted in excess during an hour after mechanical stimulation of the cervix. Like the P mucus (as will be discussed below) it may carry enzymes from the isthmus region downwards in the cervical canal. The B secretion will be discussed in more detail in a forthcoming paper.

Research during the years 1970 to 1975 indicated that the progression of spermatozoa in E mucus was complicated and it was evident that E mucus was composed of two different types of mucus, named S (-sperm-transmission) mucus and L (-locking-in) mucus because of the capacity of that mucus to attract and enclose malformed sperm (Figure 5). These results

were published (Odeblad 1977, 1978; H”glund and Odeblad 1977) and the G - L - S model was able to explain the major factors associated with the upward movement of sperm in the cervical canal.

The discovery of G, L and S mucus was presented for the first time at the University of Surrey, England, in 1976, and later at Rottach-Egern, Stockholm, New Delhi, Seattle, and in Sydney, Australia, in 1977. Most of the audiences did not understand how to apply this new knowledge. Dr Max Elstein of England readily accepted the G - L - S model but Dr Kevin Hume of Sydney, Australia, appreciated the significance of the discovery. Dr Hume was a member of the Billings group and he drew my attention to the fact that G mucus would be present in the infertile phase of the woman's menstrual cycle, and L and S types during the fertile phase, and also that S mucus would correspond with the Peak day. This was the beginning of my participation, collaboration and commitment to the Billings Ovulation Method. Following Dr Hume's recommendations, I showed for cycles of different lengths, and in women of different ages, that the agreement was statistically significant. This finding was presented at a number of conferences in several countries, towns and universities, for example in Acapulco in 1982 (published in 1983), in Melbourne in 1983 and in Paris in 1986 (Odeblad 1987).

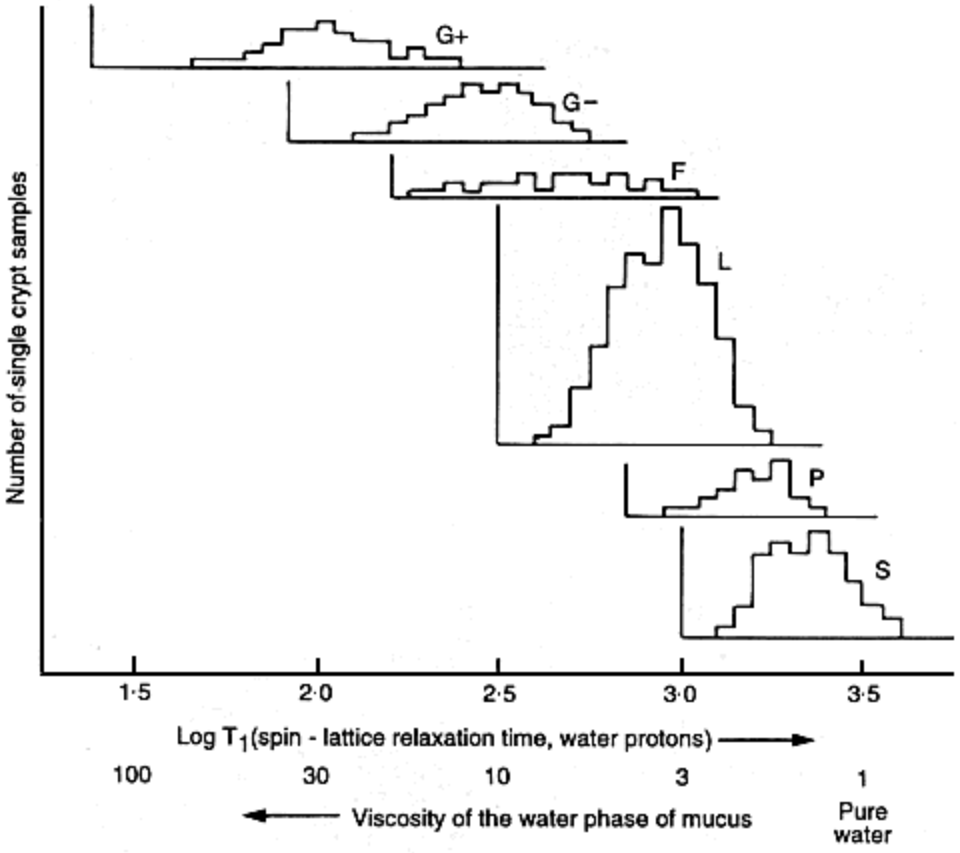
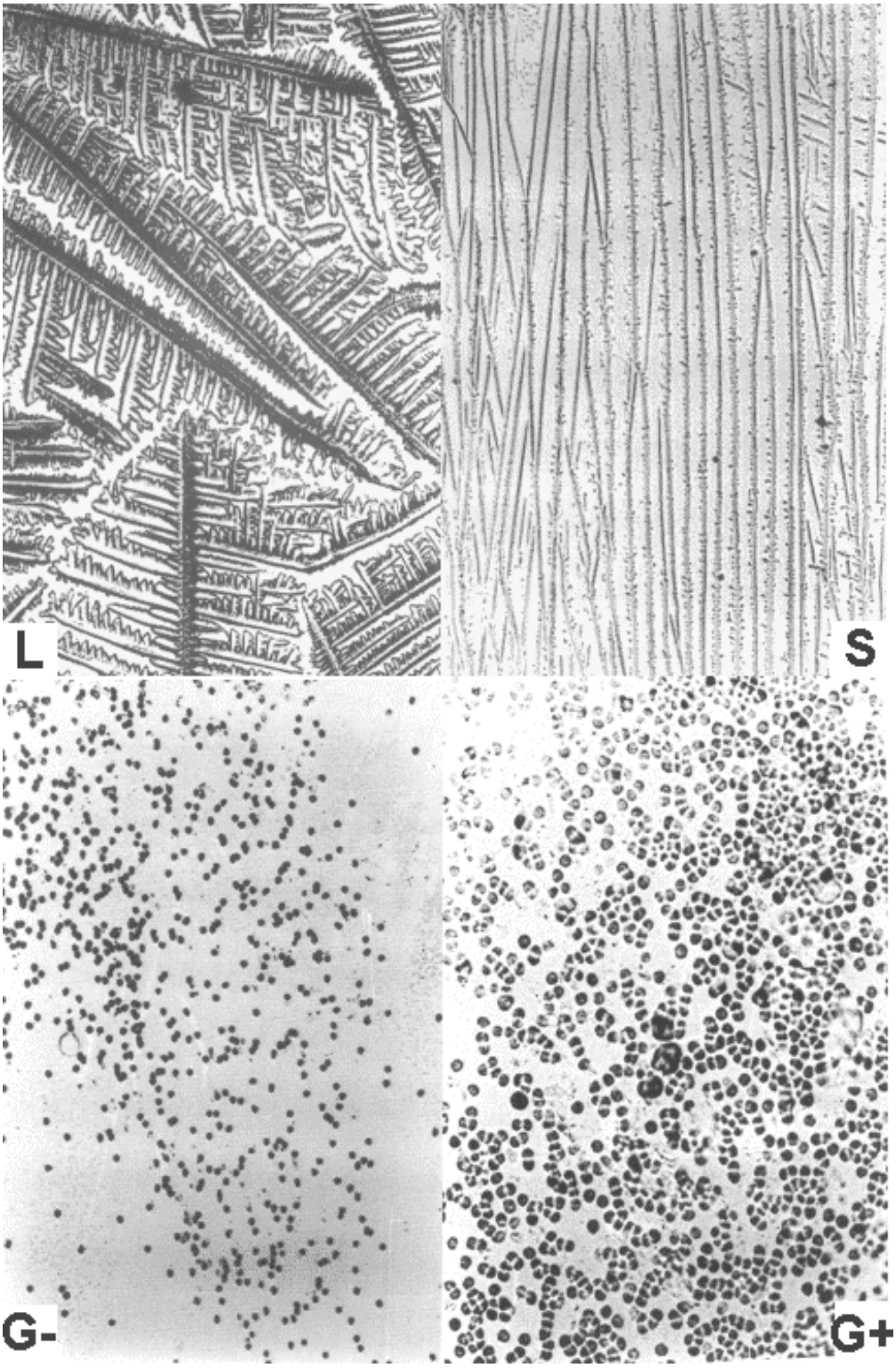


Figure 5. Viscosities of micro-samples of different types of mucus.

Studies (Odeblad *et al.* 1984) showed that S mucus was very fluid (Figure 5) and that sperm cells moved along the canal very rapidly in S mucus, reaching the S crypts in 3-10 minutes. L mucus had a medium viscosity. Unit structures of L mucus attracted malformed sperm cells or those which moved slowly, and this "filtration" of sperm cells is efficacious (Odeblad 1985). G mucus has a high viscosity and forms a sort of impenetrable plug (Odeblad *et al.* 1983).

If we take a macrosample of cervical mucus and allow it to spread out on a glass slide we are able to see with the aid of a microscope some interesting patterns (Figure 6). L mucus shows very fine crystals in the shape of rectangular leaves. In S mucus one sees crystals of another configuration -- small, thin needles. G mucus does not exhibit any crystals, but epithelial cells, leucocytes and lymphocytes. The nuclei of these cells are very abundant (Figure 7). NMR studies indicate that the water of S mucus is associated with the mucus in such a way as to form a structure which facilitates the forward movement of sperm cells (Figure 8; Odeblad 1966a).

(a)



(b)

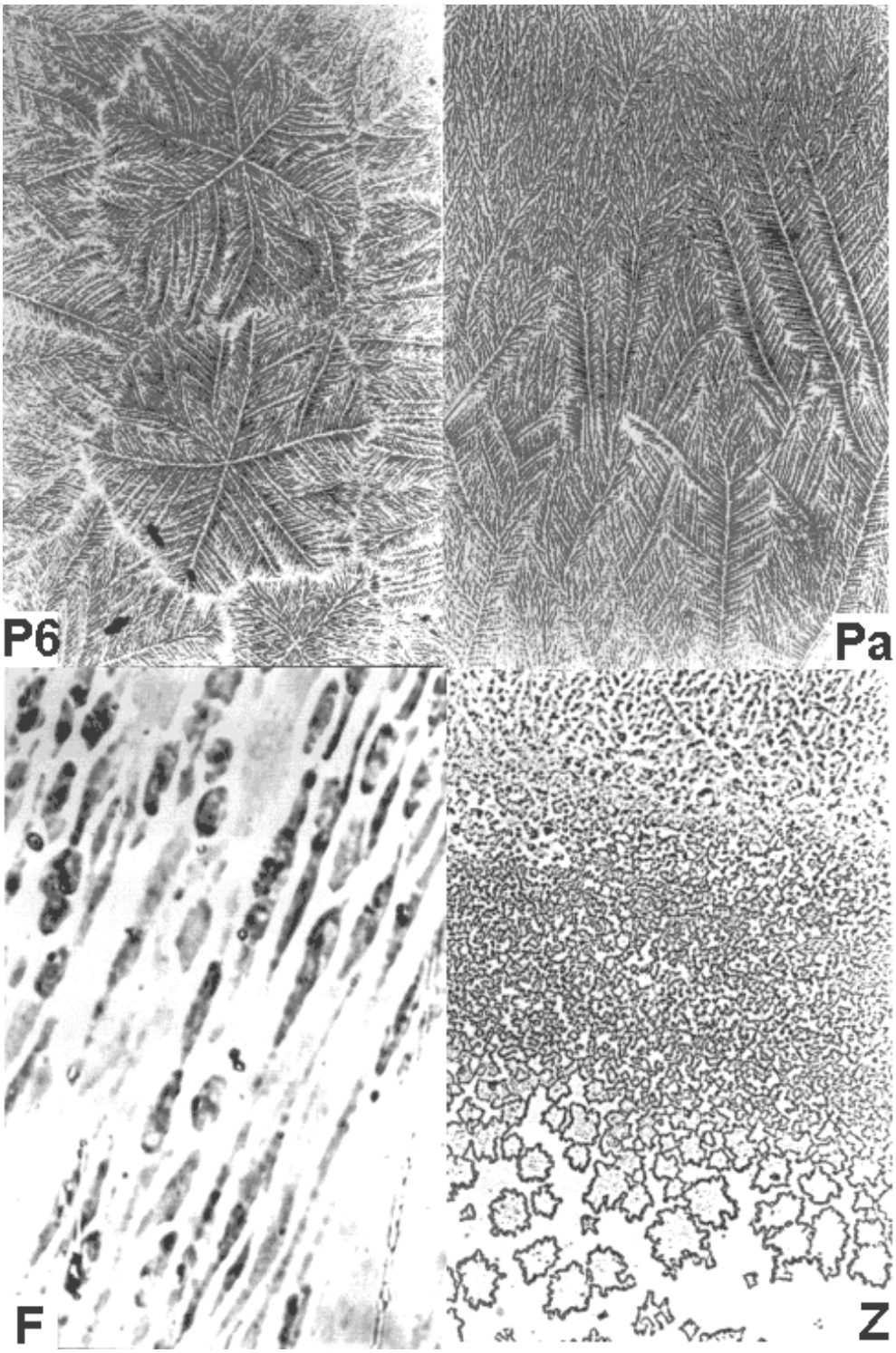


Figure 6. All mucus types present in one cervical sample obtained from a virgin woman 18 years old. Figure 6(a) shows types L (x 30), S (x 80), G- (x 80) and G+ (x 160). Note that the magnifications are all different for the different types. Figure 6(b) shows the secretions P6 (x 80), Pa (x 80), F (x 480), and Z (x 320). The F secretion contains a few leucocytes (rounded cells) among the epithelial cells (elongated) because some G secretion overlaps the F mucus. In the picture of the Z secretion one can see (to the bottom) the enzyme grains tend to aggregate into ring formed structures. To the top grains are absorbed on Pa mucus. Note that the magnifications are different for the various photos. The sample was taken the day after ovulation, approximately 17 hours after the ovulation had taken place. The woman was healthy and the large amount of leucocytes and lymphocytes in the G+ sample is a normal phenomenon.

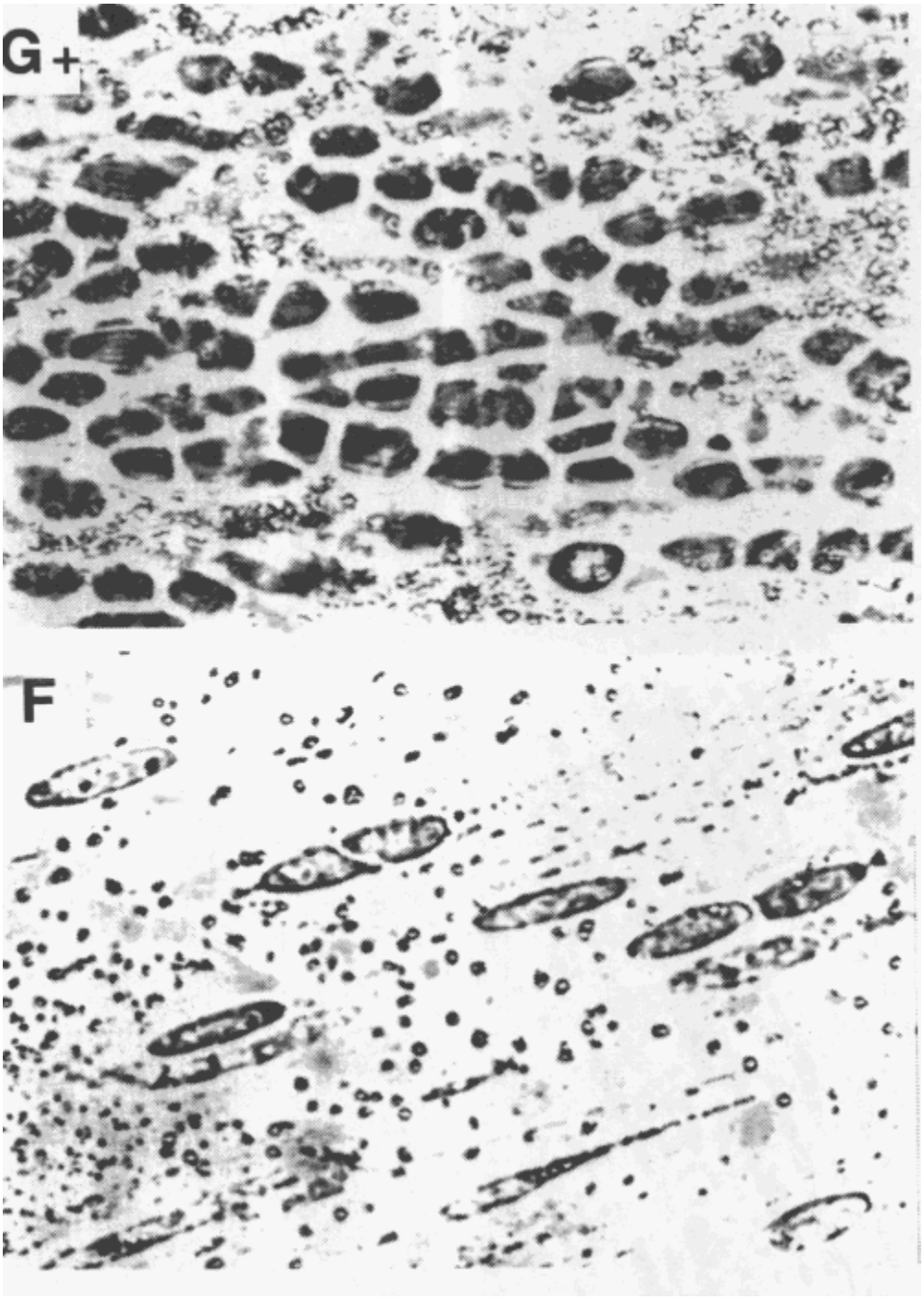


Figure 7. Comparison of G+ mucus with F mucus. G+ mucus has more leucocytes and lymphocytes. None of these cells are found in F mucus, only epithelial cells. (x 600)

In 1983 1 had the privilege of working with Drs John and Evelyn Billings in Melbourne and also with Professor James Brown and other research workers of the Ovulation Method. The hormonal response of G, L, and S mucus was studied. We found that L mucus was stimulated by medium and increasing levels, and S mucus by high levels, of oestrogen. Later I showed that S mucus was also stimulated by noradrenaline. G mucus was stimulated by progesterone. In the first infertile phase of the cycle the progesterone level is low, but sufficient to stimulate G crypts feebly (G-mucus). After ovulation, progesterone levels are high and stimulate G crypts strongly. This G mucus is very dense (G+ mucus).

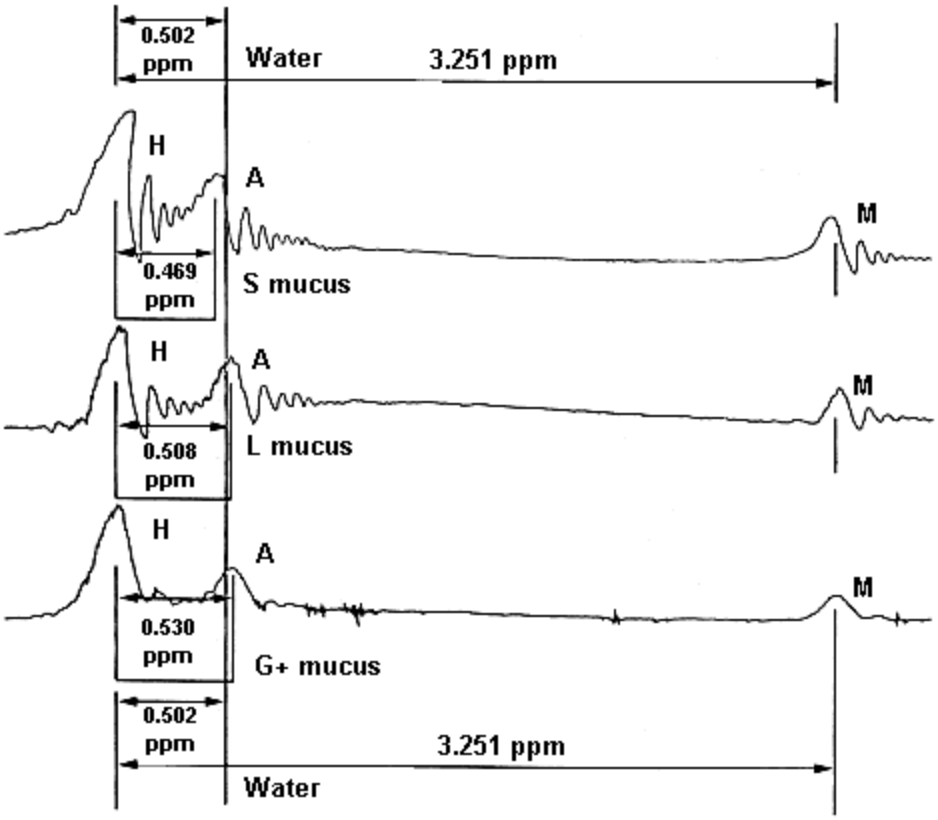


Figure 8. Enlarged NMR spectra of S, L and G+ mucus compared with that of water. This investigation demonstrated hydrogen-bonding in the aqueous phases (A) of S, L, and G+ mucus. Waves H and M are reference signals which enable the position of the aqueous signals to be obtained with very high precision. The wave shifts indicate that the water of S mucus has a small resistance to sperm cells but that of G+ mucus presents a much greater resistance.

Usually no sensation is associated with G mucus, and the days are dry during the infertile phases. When oestrogen levels increase L mucus begins to be produced, and wetness is felt, firstly with a sticky sensation. Later, when oestrogen levels are high, and S mucus is also produced, there is a slippery or lubricative sensation (Figure 15), and this sensation remains until the Peak day. On that day oestrogen levels are already decreasing but the noradrenaline-like activity of the sympathetic nervous system causes stimulation of the S mucus. Figure 15 shows the temporal relations of the different secretions. After the Peak day G mucus is accompanied by a return to a dry sensation due to the abundant secretion of progesterone by the corpus luteum.

G- and G+ Mucus

The two variants of G mucus are produced by: the same crypts, depending upon the levels of progesterone in the blood. My studies in Melbourne showed a positive correlation between the amount of progesterone and mucin content and also the number of cells in the mucus. G mucus, especially G+ mucus, probably contains antimicrobial globulins, probably the substance which occasioned the inactivation of mycoplasms in my microbiological investigations, whilst the mycoplasms were able to survive in L and S crypts which were inactive in the post-ovulatory phase.

It is important to recognize that the cells of G mucus are of three types: (i) epithelial cells; (ii) leucocytes; (iii) lymphocytes. Their proportions are variable, usually about 50% epithelial cells, 25% leucocytes and 25% Iymphocytes, but larger variations are possible, depending upon different factors. Certain women always have many lymphocytes, others have many leucocytes. Interleukins may play a role for the presence of lymphocytes and leucocytes (Cannon and Dinarello 1985). Local or general. inflammations are able to influence the proportions.

The viscosities of G- and of G+ mucus are given in Figure 5 and Table 2. The two types are impermeable to sperm cells. In non-ovulatory cycles the G+ mucus is not produced. During pregnancy, G+ is more viscous and is called Gp

mucus (p - pregnancy).

Age, Pregnancy, the Pill and Microsurgery

In young women around puberty S crypts are very numerous. Normally they are replaced by L crypts, and at premenopause the number of S crypts is considerably reduced. This transformation of L → S crypts is a normal process. There is also a G → L transformation. Also some columnar secretory cells on the portio are replaced by stratified epithelium which advances in a centripetal manner towards the cervical orifice. The L → S and G → L transformations are partially reversed by changes during pregnancy, but they are partially accelerated by the Pill. These circumstances may be simply stated by the expression: a pregnancy rejuvenates the cervix by 2-3 years, but for each year the Pill is taken, the cervix ages by an extra year.

If a woman takes the Pill for 10-15 years and then ceases taking it in order to achieve pregnancy, she may encounter some difficulties. Studies indicate that the number of S crypts are very few and, as well, the cervical canal will be very narrow. In such cases I have attempted to imitate, by microsurgery, the rejuvenation which normally occurs during pregnancy, that is to allow S cells to "take over" the L crypts. Sometimes, in about 40% of cases, this microsurgery was not successful.

P Mucus

Examination of microsamples of crypts, the cells of which were not able to take over an L crypt, presented some new results. The crystals were not needle-shaped nor did they have rectangular branching. The branching was hexagonal (Figure 9) and the crystals were very thin. Re-exaniination of the slides obtained during complete cycles indicated that this mucus was usually present in maximum amount on the Peak day. The new mucus was called P (- peak) mucus.

In the years following 1985 several young women 15-22 years old who were students at the university or high school wished to be taught how to chart their cycles. Most of them also wished to have a gynaecological examination and their mucus was placed on slides and examined. I was astonished at the large amounts of P mucus in these young, women. I had often observed that a large area of the slide was covered with P mucus, especially on the Peak day and. the day before the Peak. In these cases the women described their sensation as extremely lubricative during these same two days.

After the P mucus was identified and characterized in 1990 I found, on reading medical journals, that crystals of this mucus had already been photographed by several authors, for example Roland (1958) and again by Rydberg (1948), the one who first described the crystallization of mucus. In 1991, I found (see Odeblad 1994) that P mucus had two functions: (i) a mucolytic activity, variant Pa; (ii) a capacity to conduct sperm cells from the S crypts to the uterine cavity, variant P6. These two variants are illustrated in Figure 9.

I found that the mucolytic activity is effected by an enzyme which is associated with granules (spheres) about 1μm in size (cf. Figures 6b and 10) which adhere to the P mucus.

Also the granules have the capacity to aggregate and form annular or stellate shapes (Figure 6b). This granular secretion is termed Z secretion (cf. z in the word enzyme).This secretion which is produced by the glands (or crypts) of the isthmus, probably does not vary during the cycle, and may contain other enzymes or biochemical substances as well. Recent studio indicate, however, that Z cells may also be present in the cervical canal proper.

Proton NMR spectroscopy indicates that the molecular composition of the various types of mucus are different (Figure 11). Studies of microsamples obtained from different locations in the cervical canal demonstrate that the various types of crypts have "preferred locations" (Figure 12). Both the macromolecular networks and the crypt locations are thus specific for each type of mucus.

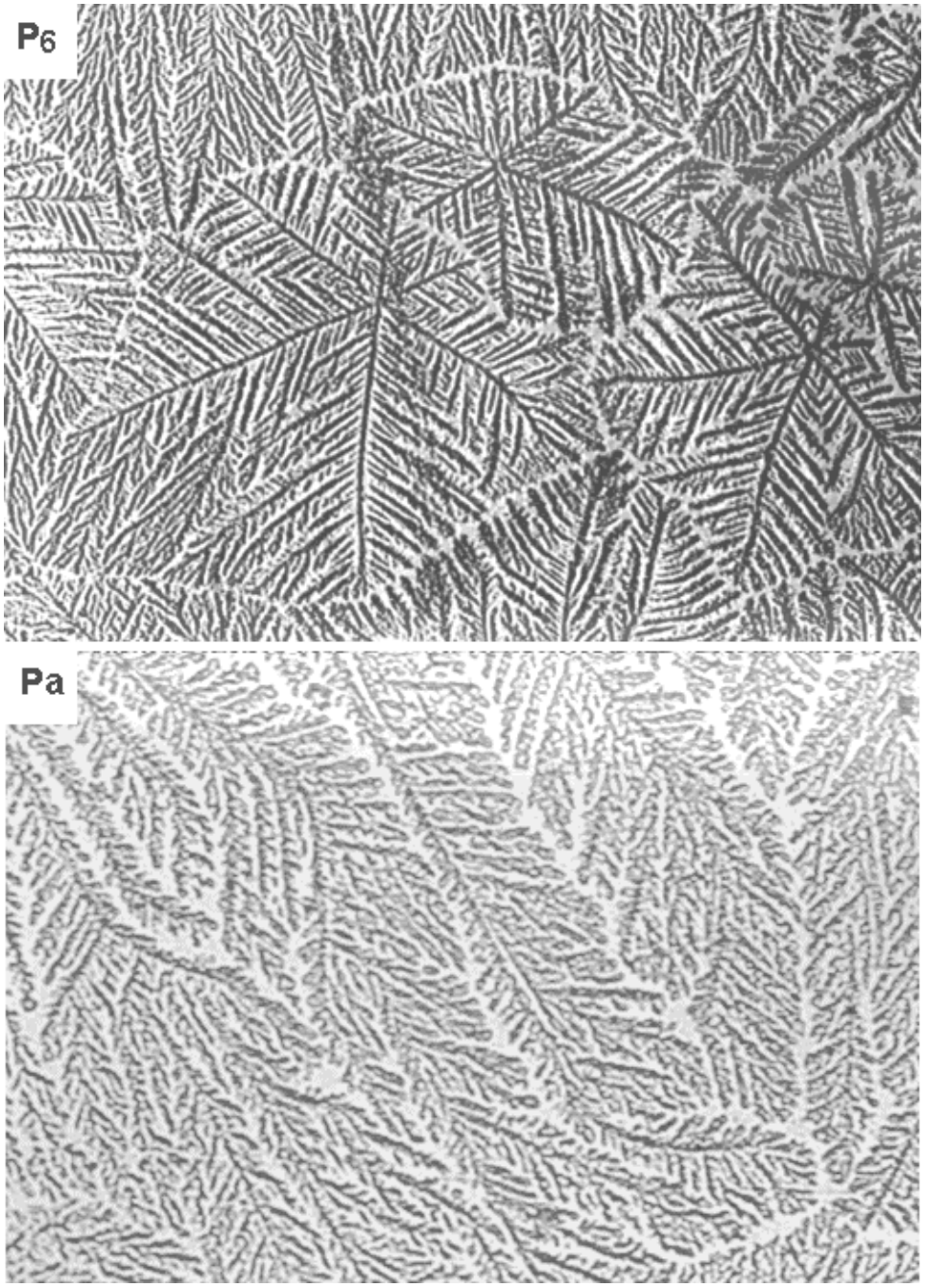


Figure 9. Comparison of P6 mucus with Pa mucus in a sample spread out on a Microscope slide. In P6 mucus the crystals are present in hexagonal star-shaped formation. In Pa mucus crystals are present in plume-shaped formation or are irregular in shape.

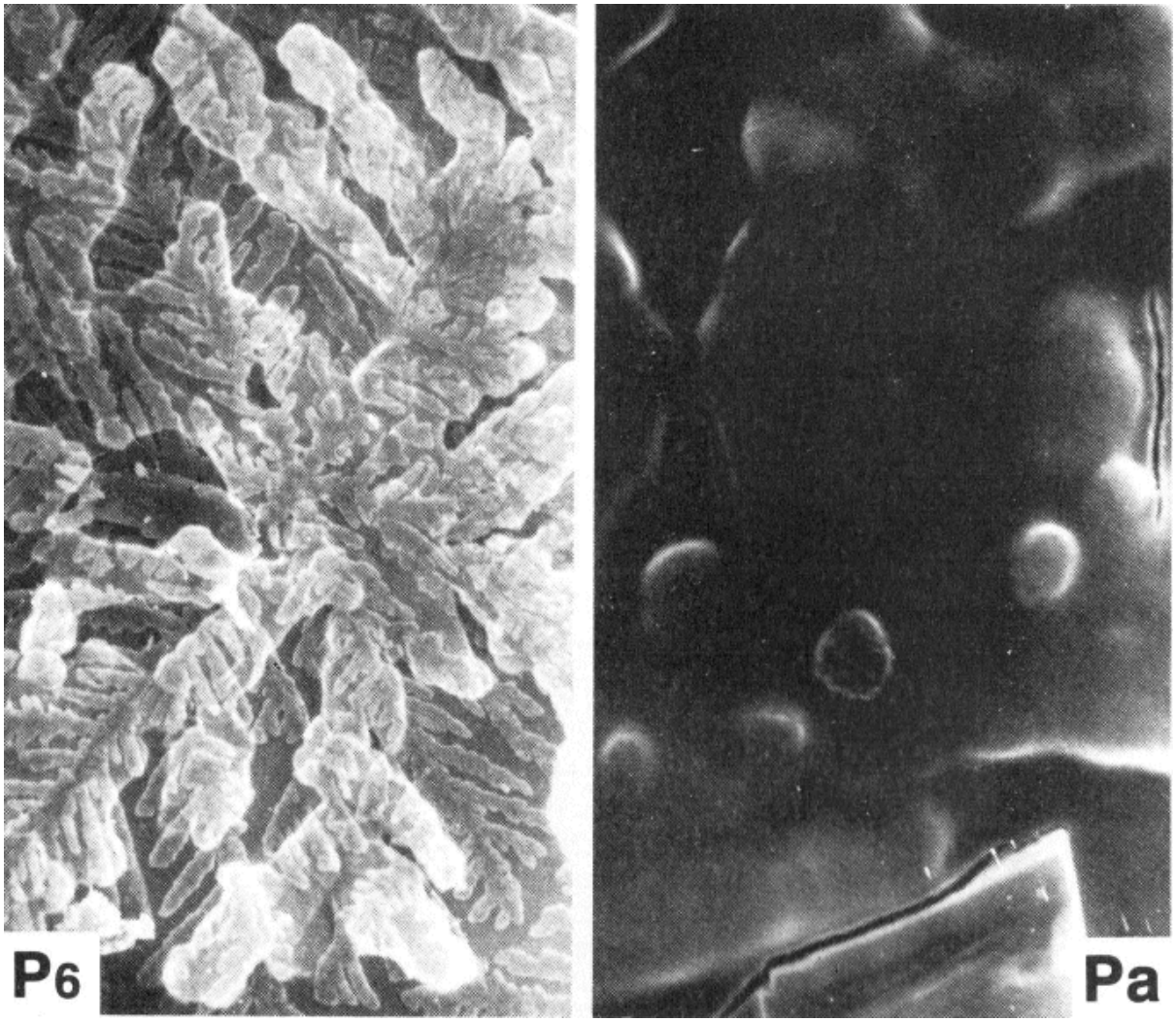


Figure 10. Scanning electron microscope pictures of a sample of P6 mucus (left) and Pa mucus (right) spread out on a microscope slide. P6 mucus shows hexagonal branches and some granules (round, white spheres). x 1000. Pa mucus shows some granules (round, dark spheres). x 7500. Pictures taken by Dr Mikaela Menarguez, Department of Cellular Biology, University of Murcia, Spain, in collaboration with the author.

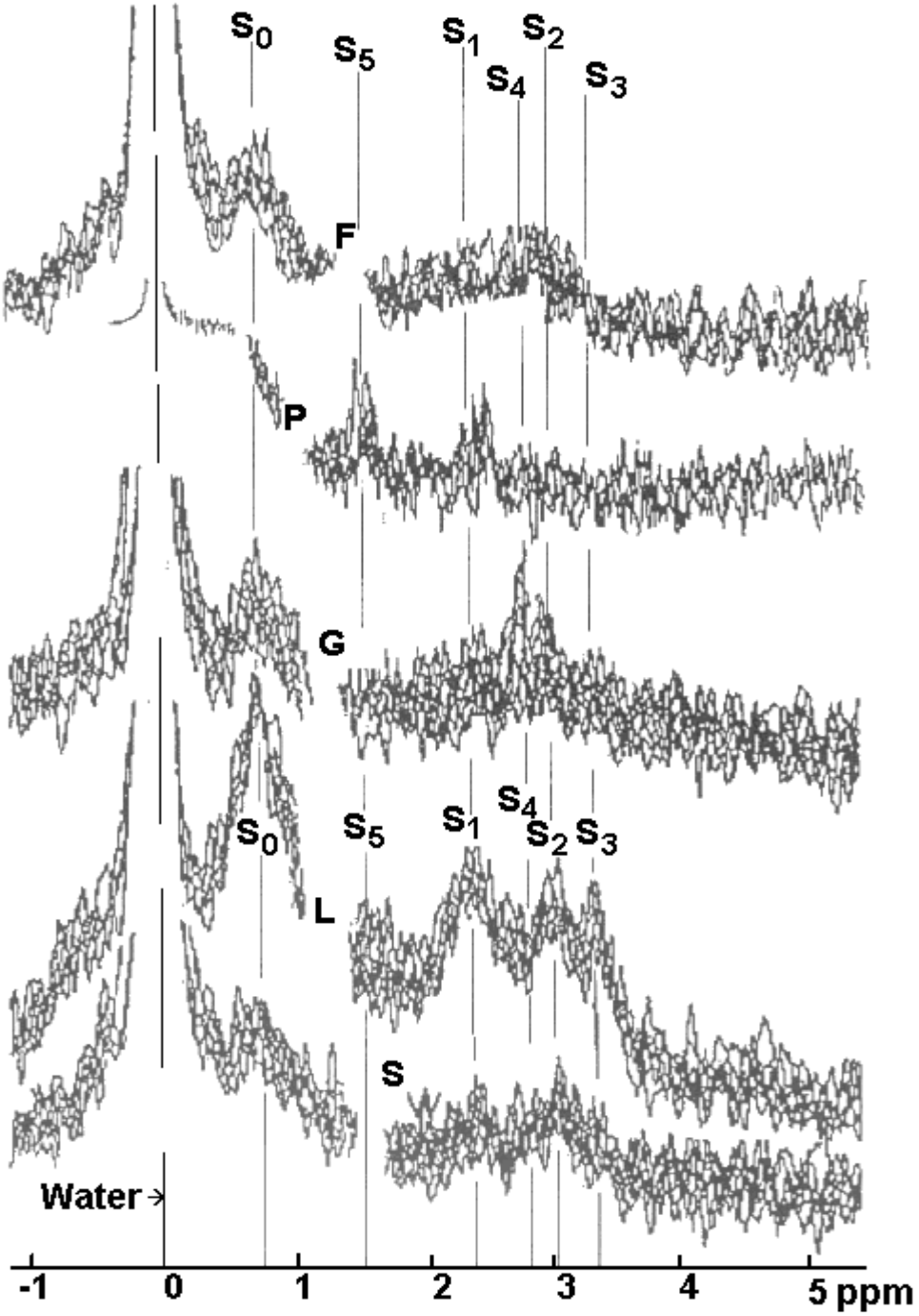


Figure 11. Proton NMR spectra of five types of cervical mucus - F, P, G+, L and S. All spectra are different, indicating that the compositions of the five mucus types are all different.

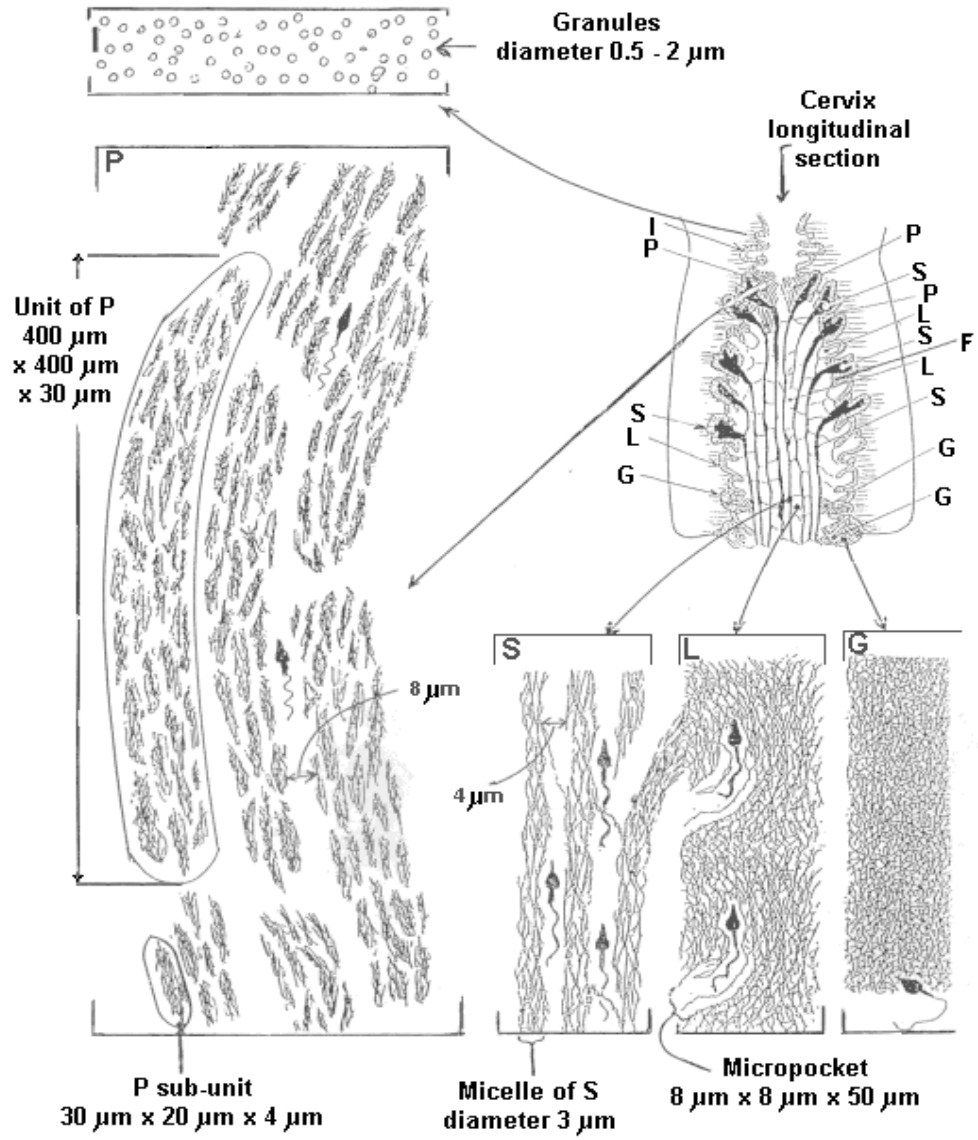


Figure 12. Distribution and macromolecular composition of cervical mucus types S, L, G and P, and the Z secretion from the isthmus.

F Mucus

A detailed analysis of about 600 charts, several kept over a period of 2-4 years (totally more than 12,000 cycles), showed that several young women normally charted a number of days with yellow stamps ("yellow days") in the infertile phases, despite the fact that their gynaecological examinations were perfectly normal. Most of these young women were virgins who had never experienced any genital infection. A careful study of their slides showed the existence of a mucus which resembled G- or G+ mucus, but leucocytes and lymphocytes were very rare. Precise aspiration of this mucus showed that it was produced not in the crypts but in the epithelium covering the endocervical wall between the openings to the crypts (Figure 1).

The cells which cover the walls of the canal are probably more original or fundamental than the cells which differentiate in the crypts. This assumption is sustained by the observation that the younger the woman the more abundant is the quantity of mucus of this type. I have called this secretion F (- fundamental) mucus and the cells F cells.

The description of the mature cervix and crypts, shown in Figures 1 and 12, is valid for the fertile years of the healthy woman. To understand the mature cervix it is important to study the development of the cervix during embryonic life, during infancy, adolescence and also its regression during and after the menopause.

Reports on embryonic development are plentiful (e.g. Davies and Kusuma 1962; Copplesson *et al.* 1967; Hiersche 1970;

O'Rahilly 1973; Forsberg 1976; Graham 1976; Pixley 1976). The following description is perhaps admissible.

In an embryo 8-12 mm long (Figure 13a) the urogenital organs are rather similar in both sexes. On each side in the abdominal cavity there is a fold, the urogenital fold (UGF) containing two ducts (from the beginning solid cell strings), called the Wolffian duct (W) and the Müllerian duct (Mü). The Wolffian duct is localized nearer the mid-plane of the body and has three groups of horizontal ramifications to three primitive organs denominated the pronephros (P), the primitive gonad (Ov) and the metanephros (K). The pronephros soon disappears completely, the primitive gonad develops. into a testicle or an ovary. The metanephros develops into the kidney and is displaced upwards, while the gonad is displaced downwards. This downward movement of the gonad is supported by a fibrous structure called the gubemaculum (Gu). The testicle descends considerably through the inguinal canal to the scrotum, while the ovary only descends a little, but retains a fibrous connection via the gubemaculum through the inguinal canal with blood and lymph vessels.

In the male the Müllerian ducts undergo regression while the Wolffian ducts develop into the vas deferens and the epididymis. In the female the Wolffian ducts undergo regression and the Müllerian ducts develop into the tubes, the uterus (with the cervix) and the upper part of the vagina. Remnants of the Wolffian ducts may, however, be present in some women.

In the female embryo the gubemaculum and the Müllerian duct cross each other (at x in Figure 13a) and they adhere to each other at the point of crossing. The Müllerian duct above the crossing point becomes the tube. Below the crossing point the two Müllerian ducts fuse to one single structure and form the uterus and the upper part of the vagina. The lower part of the vagina (including the pockets of Shaw) develop from the urogenital sinus (UGS).

The fused part of the Müllerian ducts is from the beginning a solid cell string, but it liquefies centrally and forms the cervical canal with its epithelial lining. Glandular structures are visible in the cervix 2-3 months before birth. The epithelium of the cervix is, however, very different from the mature mucous membrane. Before birth it is composed of two or more layers of cells (Figure 13b). The superficial cells have the appearance of the secretory cells of the mature cervix and a secretory activity can be demonstrated by histochemical methods (Nonnis-Marzano and Zinelli 1959). The basal cells are clear and transparent. Reid *et al.* (1967) have proposed that these cells may be derived from monocytes which traverse the capillary walls and move to their epithelial positions.

Clear or transparent cells are subject to regression and finally they are found in certain locations only. The physiological meaning of this distribution is not known to this day. My suggestion is that these islands of basal cells produce inductor substances leading to a differentiation of F cells into P, S, L and cells (Figures 13a and 13b).

The first secreting structures may be designated primordial G, L, S or P crypts. Later they are stimulated hormonally to develop into mature G, L, S and P crypts (Figure 13b). A transient stimulus, which depends on the production of placental oestrogen, takes place in the last month before birth. After birth, this stimulus ceases and the crypts atrophy, "the genital crisis of the newborn" (Courrier 1945). New proliferation commences before and during puberty. At first, L, S and P crypts are stimulated; after several years G crypts are stimulated by progesterone, when ovulatory cycles commence.

The mechanisms whereby F cells are transformed into G, L, S or P cells are still unknown. Probably an understanding will be reached by molecular biology studies by the introduction of transparent fetal cells into the groups of F-cell culture *in vitro.*

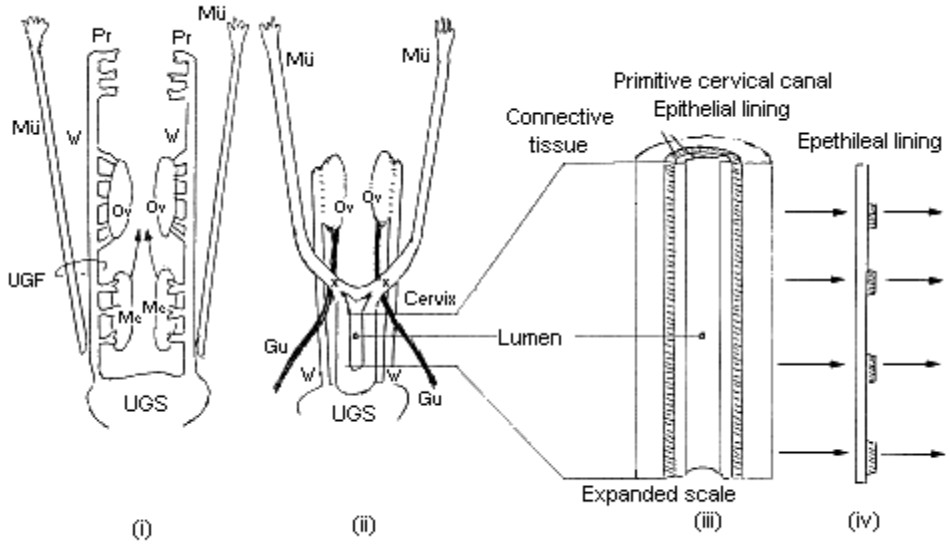
During infancy, the mucous membrane of the cervix is flat or folded or sometimes lumpy, but few crypts are present. It produces only minimum quantities of mucus. The cells are probably more frequently F cells. Similarly, after menopause, differentiated cells are unusual and F cells are more frequent, the differentiated G, L, S and P cells have exfoliated or degenerated.

F mucus is probably made up of a large excess of membranous glycoproteins of F cells which are exfoliated and visible in the mucus. Leucocytes and lymphocytes are rare. Crystals of F secretion are few and indistinct or they are not present at all, and usually the nuclei of F cells are only visible on slides after the F mucus has dried out. Often minimum quantities of F mucus are mixed with G, L, S or P secretions and L, S, and P crystals are altered and form irregular patterns. In women of reproductive age F mucus amounts to 1-4% of the total cervical mucus (Figure 14b).

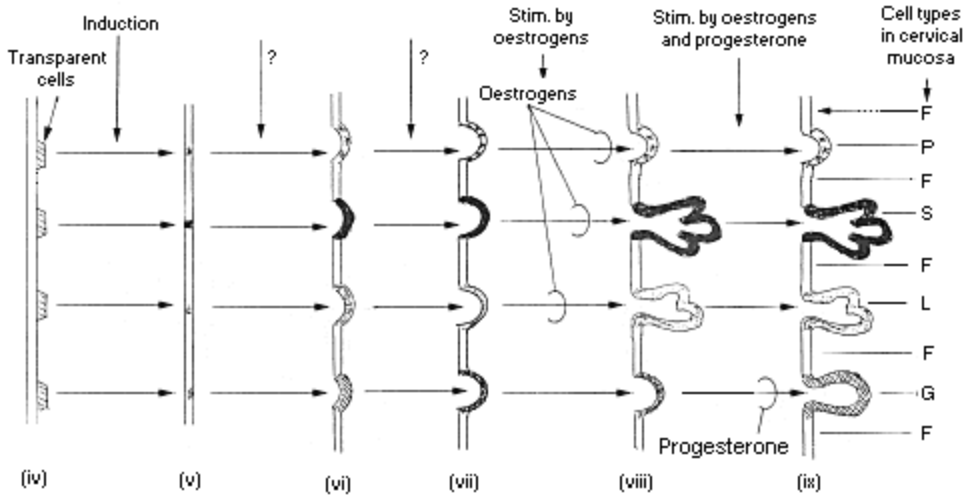
It was propounded already by Jost (1947) that there could be a factor, later denominated the Müllerian inhibition substance (MIS), or Jost factor, which aids in the regression of the Müllerian duct in the male fetus. Later studies (Takahashi *et al.* 1986; Vigier *et al.*1989) have shown that MIS is also present in the female, and may inhibit oocyte meiosis at puberty and reduce ovarian aromatase activity, a biochemical step in the biosynthesis of oestrogens. In male fetuses and young male infants MIS promotes the descent of the testes into the scrotum. Biochemically MIS appears to be a glycoprotein with a general "anti-Müllerian" and "pro-Wolffian" activity. Functionally it is classified as transforming growth factor beta

(TGF-β).

(a)



(b)



Figures 13(a) and 13(b) indicate schematically the development of the cervical crypts. Figure 13(a) covers the period from embryo to mid- gestation and Figure 13(b) from mid-gestation up to the period of late puberty and adulthood. The stages are (i) embryo 8-12 mm in length, 35-40 days old; (ii) embryo 27-33 mm in length, 50-55 days old; (iii) longitudinal section of (ii) on an expanded scale; (iv) fetus 4-5 months of age; (v) fetus 1-2 months before birth, induction complete; (vi) 2-4 months after birth - primordial crypts; (vii) childhood secondary crypts; (viii) beginning of asolescence: P, S, L crypts develop; (ix) end of adolescence, maturity. G crypts also develop. Stages (v) to (vi) and (vi) to (vii) may be stimulated by the hormones GH, EGF and thyroxine. The code for the shading indicating the different types of cells present is given at the right hand side of Figure 13(b). Mü, Müllerian ducts; W, Wolffian ducts; Pr, pronephros; Ov, primitive ovary; Me, metanephros (which become the kidneys); UGF, urogenital fold; UGS, urogenital sinus; x, crossing point of gubernaculum (Gu) and Müllerian ducts.

I have suggested that MIS might be involved in the development of the "missed mucus symptom", a rare but interesting disease which was first publicly presented at the 1990 Annual Meeting of the Natural Family Planning Council of Victoria in Melbourne, Australia. Women with this condition often seek help for infertility. Their charts reveal that they sometimes

miss their mucus symptom, apparently due to a temporary closing of the external os. This in turn is due to contraction in the two sacro-uterine ligaments which contain muscle bundles stimulated to contractions by oestrogenic hormones. The tension at contraction is mediated to the region of the external os by fibrous strings, remnants of the Wolffian ducts, one on each side of the cervix. These women may have other signs of "anti-Müllerian" activity such as a small amount of cervical mucosa and impaired ovarian function.

The Role of the Vagina

Not only the cells of the cervix but also the cells of the vagina produce a mucus which is made up of membranous glycoproteins. In the vagina there are intermediate cells which are able to contribute to the presence of mucus on infertile days. A reason for the very frequent appearance of a flow outside the vagina during those days is a reduction of the reabsorption by the pockets of Shaw (Figures 1 and 14). The pockets are situated in the inferior part of the vagina and the contribution of vaginal mucus emanates from the superior part. In this case a gynaecological examination is quite normal and cytological examination shows more intermediate cells. Examination of a slide by microscope also shows quantities of F mucus in the cervical "spread out" specimens.

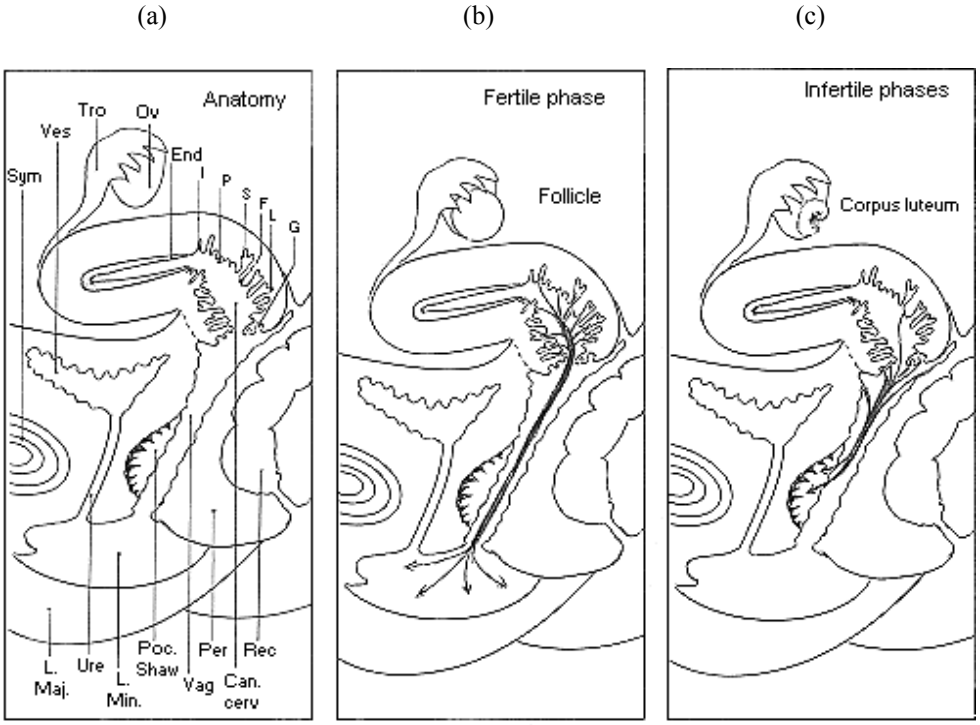


Figure 14. Cervical anatomy and mucus and fluid flow during fertile and infertile phases of the cycle. The locations of crypts P, S, L, and G are indicated as also are F cells (F); I, isthmus (secretion Z); Can cerv, cervical canal; End, endometrium; L. maj., Labium major; L. min., Labium minor; Ov, ovary; Per, perinium; Poc. Shaw, pocket of Shaw; Rec, rectum; Sym, symphesis; Tro, tube; Ure, urethra; Vag, vagina; Ves, bladder.

Normally the reabsorption by the pockets of Shaw is reduced in the fertile phase because of the thickening of the preovulatory vaginal epithelium (Odeblad 1989). The capacity for reabsorption during adolescence may be retarded and dry days may be absent. If a young woman commences to take the Pill, maturation of the pockets is even more retarded. Forthcoming papers will give more detailed presentations of the role of the vagina for the Ovulation Method.

The Different Types of Secretions and the Billings Ovulation Method

The most important characteristics of the five types of mucus secretions are given in Tables 1 and 2. The secretions of the glands of the isthmus are also given in these tables. I would now like to explain the normal ovulatory cycle on the basis of our knowledge of these types of cervical mucus (Table 3). I shall illustrate the situation in general for mature women 23-37 years of age and shall explain by examples the situation for young women 13-22 years of age and for women approaching the menopause (38-47 years), and also draw your attention to some factors during lactation.

Table 1. Cells detected in, and crystalline structure of, the various mucus secretions

0, not present; (+), present sometimes in small amount; +, present in small amount; ++, moderate amount; +++, large amount

Cells and crystals observed in mucus	Mucus						
	G-	G+	L	S	P6	Pa	F
Cells							
Leucocytes	++	+++	(+)	0	(+)	(+)	(+)
Lymphocytes	++	+++	0	0	0	0	(+)
Epithelial cells	++	+++	0	0	(+)	(+)	(+)
Sperm cells 12-24 hr post coitum	0	0	+	++	+	0	0
Enzyme granules	+	+	+	+	+	+++	+
Crystals							
Rectangular	0	0	+++	0	0	0	0
Hexagonal	0	0	0	0	+++	+++	0
Parallel needles	0	0	0	+++	0	0	0
Irregular	(+)	(+)	0	0	0	+	(+)

Table 2. Some physical, chemical and biological properties of the various cervical secretions and secretions of the isthmus and the vagina

Mucus characteristic	Mucus type			
	G-	G+	L	S
Place of biosynthesis in cervix	Lowest third	Lowest third	All cervix	Upper half
Hormonal and other stimulation	Low progesterone	High progesterone; interleukin 1	Averzge and increasing levels of oestrogens	High oestrogens; noradrenaline
Average viscosity by NMR	11	30	3.5	1.3
(95% interval)	(6 to 17)	(15 to 45)	(2.4 to 5)	(0.9 to 2.2)
Apparent viscosity	High	Very high	Medium	Fluid
Function in ascent of spermatozoa	Barrier to sperm advancement	Barrier to sperm advancement	Attracts malformed sperm	Conveys normal sperm to the crypts
Presence during cycle	First infertile phase	Second infertile phase	Fertile phase	Pre-ovulatory phase and Peak day
Sensation at the vulva	Dry	Dry	Wet, sticky	Wet, lubricative
Approximate time for mucolysis	24 hr	36 hr	5 hr	5 hr

Table 2 (cont'd)

Mucus characteristic	Mucus type				
	P6	Pa	F	Z	"Vaginal mucus"
Place of biosynthesis in cervix	Upper fifth	Upper fifth	All cervix	Isthmus	Vagina
Hormonal and other stimulation	High and decreasing oestrogens; noradrenaline	High and decreasing oestrogens; noradrenaline	Probably none	Probably none	Low and average levels of oestrogens;
Average viscosity by NMR (95% interval) Apparent viscosity	2.0 (1.4 to 3) Fluid	2.0 (1.4 to 3) Fluid	7 (2 to 11) Medium	1.5 (1 to 6) Commonly Fluid	5 (2 to 10) Low or medium
Function in ascent of spermatozoa	Conveys normal sperm from crypts upwards	Absorbs Z secretion and performs mucolysis	No known function	Various enzymatic activities	-
Presence during cycle	Beginning and end of the fertile phase	Beginning nd end of the fertile phase	Throughout cycle	Probably throughout cycle	Infertile phases
Sensation at the vulva	At Peak day wet and very lubricative	Loosens plug. Wet and very lubricative	Sticky	Wet	Flaky
Approximate time for mucolysis	1.5 hr	1.5 hr	24 hr	-	36 hr

Early Infertile Days

After menstruation there are usually some days of dryness in the genital parts outside the vagina, and green stamps are used for the record. G mucus and minimal quantities of F mucus and of vaginal fluid are produced (Figure 15). They are viscous and do not flow rapidly in the vagina. Active reabsorption takes place in the pockets of Shaw and all factors contribute to a sensation of dryness. Sometimes, in the young woman, F mucus is very abundant and as the reabsorption processes have not yet developed a crumbly or sticky sensation of dryness is felt and yellow stamps are used. In the older woman a similar situation prevails, since reabsorption is diminished after the menopause. Not only F mucus but also vaginal contributions lead to the use of yellow stamps. The term "Basic Infertile Pattern" is used by Billings *et al.* (1989) for the situation during the first infertile phase before follicle maturation begins and in other situations with absent or delayed follicular growth. On p. 21 of that publication it is stated that the Basic Infertile Pattern may be either (i) dry days, indicated by green stamps, (ii) an unchanging continuous mucus pattern (yellow stamps), or (iii) a combined pattern (green stamps for dry days, yellow stamps for unchanging, continuous mucus days). In that same publication (Odeblad 1989) some considerations are given on the combined role of the vagina and the cervix for vaginal discharge. In a forthcoming paper these considerations will be extended in the light of the new discoveries of the P mucus, the mucolytic factor, the F mucus and B secretion.

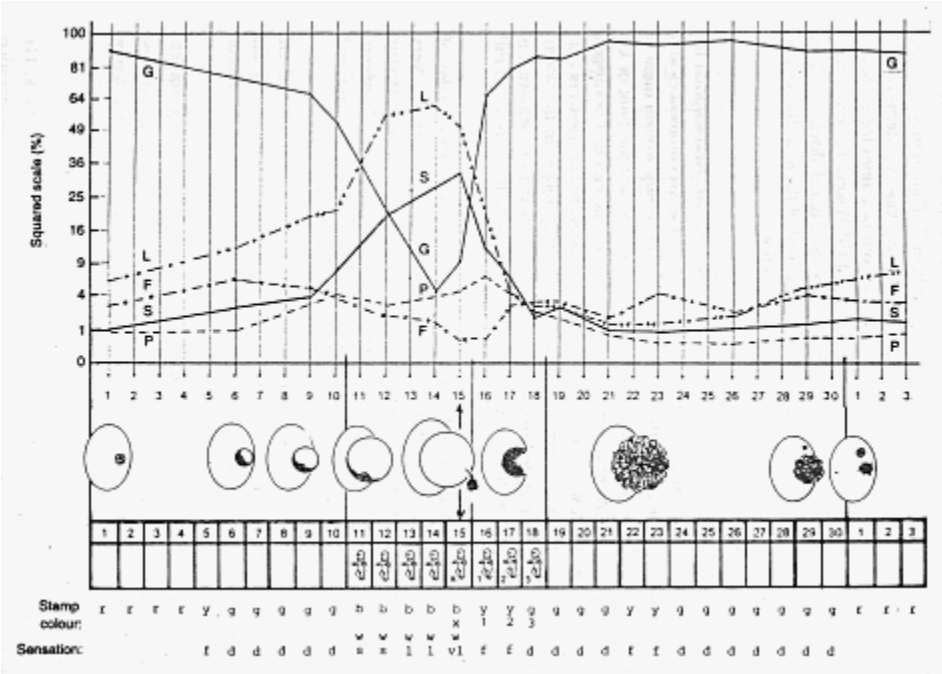


Figure 15. Cycle of a high-school student, a virgin 15 years of age. She suffered a slight haemorrhage and treatment with trenexamic acid (*trans*-4-aminoethyl-cyclohexane carboxylic acid) remedied the problem. She had been charting for several years. Analyses of types of mucus (S, L, G, P, F) on a microscope slide are given. Day of ovulation was determined by repeated ovarian palpation. This cycle has a short mucus phase (5 days) but was within normal limits (5 - 10 days) as indicated in Table 3. Stamp colours r, red; y, yellow; g, green; b, white stamp with baby imprint (mucus days); x = Peak day; days 1 and 2 after peak, yellow stamp with baby imprint; day 3 after peak, green stamp with baby imprint. Sensation: d, dry; w, wet; s, sticky; l, lubricative; vl, very lubricative.

The Days of Possible Fertility

Increased oestrogen levels elicit a secretion of mucus in the L crypts. Reabsorption by the pockets of Shaw is reduced (Figure 14) and a moist, sticky sensation commences and white baby stamps are used on the chart. Often and especially with young women there is a secretion of P mucus which has a mucolytic activity (the granules) and the plug of G mucus is dislodged and expelled. After 1-3 days there is a change, the elevated oestrogen levels stimulate the S crypts and a wet and slippery sensation (Odeblad *et al*. 1986) is felt, depending on the presence of L and S mucus. This phase is usually longer in the young woman (Table 3) and also with a mature parous woman. The wet and slippery sensation is much shorter in women approaching the menopause and in women who have taken the Pill for several years.

Table 3. The phases of normal ovulatory cycles explained in terms of quantities of cervical mucus secretions for women aged between 13 and 22, 23 and 37, and 39 and 47 years of age
(+), 0.5 to 1.5%; 0, <1%; +, 2 to 6%; ++, 7 to 30%; +++, >30%*

Phase of cycle	Mean length (days) (95% interval)	Mucus present (%)				
		P	S	L	F	G
Women aged 13 - 22 years						
Menstruation	4.5					
First infertile days	8 (2 to 13)	0	0	+	+	+++
Days of possible fertility	7.5 (5 to 10)					
Start (days moist and sticky)		++	0	++	0	0, +**
|		0	+	+++	0	0
|		+	++	+++	0	0
Peak day (very slippery)		++	++	+++	0	0
Day 1 post peak	1	+	(+)	+	(+)	++
Day 2 post peak	1	0	0	0	0	+++
Day 3 post Peak	1	0	0	0	0	+++
Late infertile days	8 (6 to 14)	0	0	0	+	+++
Whole cycle	31 (22 to 41)					
Women aged 23 - 37 years						
Menstruation	4					
First infertile days	6 (4 to 10)	0	0	+	0	+++
Days of possible fertility	6 (4 to 8)					
Start (days moist and sticky)		+	0	++	0	0, +**
|		0	+	+++	0	+
|		0	++	+++	0	0
Peak day (very slippery)		+	+++	+++	0	0
Day 1 post peak	1	+	0	+	0	+++
Day 2 post peak	1	0	0	0	0	+++
Day 3 post Peak	1	0	0	0	0	+++
Late infertile days	10 (8 to 14)	0	0	0	0	+++
Whole cycle	29 (23 to 36)					
Women aged 38 - 47 years						
Menstruation	5.5					
First infertile days	5.5 (2 to 9)	0	0	0	+	+++
Days of possible fertility	3.5 (1 to 5)					
Start (days moist and sticky)		0	0	++	0	+
|		0	0	+++	0	0
|		0	+	+++	0	0
Peak day (very slippery)		+	++	+++	0	0
Day 1 post peak	1	0	0	+	0	+++
Day 2 post peak	1	0	0	0	+	+++
Day 3 post Peak	1	0	0	0	+	+++
Late infertile days	10.5 (8 to 16)	0	0	0	+	+++
Whole cycle	28 (22 to 34)					

Footnotes to Table 3:
* The semi-quantitative notations 0, (+), +, ++, +++ have the same meaning as in Table 1, namely 0, not present; (+), present sometimes in small amount; +, present in small amount; ++, moderate amount; +++, large amount. They are of importance for the doctor's diagnosis of, for example, the cause of infertility. For the woman, charting the qualitative sensation at the vulva forms the important observations which are notd using coloured stamps (see Figure 15) and supplementary wording.

** Sometimes a plug.--

The last day of the slippery sensation is the Peak day, which coincides with the day of ovulation in 80% of cases, and the probability of conceiving is highest on that day. It is very important to know that the quantity of mucus is usually not at its

maximum on the Peak day.

The quantity and also the stretchiness are greater on the day preceding the Peak. An exception is the older woman. For her the fertile phase is very short, only one or two days, and in this case, the quantity and the stretchiness are maximal during the Peak day.

In the young woman P mucus increases afresh about the time of ovulation and the mucolytic activity is augmented. There are two effects of this phenomenon: (i) the lubricative sensation is increased; (ii) if pregnancy is desired, there is lysis of the mucus above the S crypts and sperm cells move upwards to the uterine cavity.

Sometimes this augmented lubricative sensation is present for 2 days in young women.

After the Peak day there are 3 days of possible but decreasing fertility. Green baby stamps are used on the chart unless some traces of discharge are present when yellow baby stamps are used.

Late Infertile Days

The late infertile phase (Figure 15) commences on the fourth day after the Peak. G+ mucus is produced and forms an impenetrable barrier in the cervix. Usually the sensation is one of dryness and green stamps are used. Normally there is a preponderance of G+ mucus and much less F mucus in the cervix, and vaginal mucus secretion is minimal. There is active reabsorption by the pockets of Shaw and the situation is very similar to the first infertile phase. On rare occasions some sticky mucus or vaginal discharge may flow and a yellow stamp is applied to the chart. On the first day after the Peak it is usual to find on a slide mucus types G, L, S, P and F, and also secretion Z, especially in young women (Table 3).

Anovulatory Cycles

These cycles vary enormously. Often there is a regression of a follicle for a week and cervical secretion resembles the situation on the first infertile days. On the day after the follicle has reached its maximum size all types of mucus are visible on a microscope slide (Figures 6a and 6b). Sometimes the follicle persists for 2-3 weeks before regression commences and at other times the regression is very rapid 2-3 days before menstruation.

Lactation

Some months after birth a very small number of follicles begin to grow but they do not reach maturity. In these attempts, waxing and waning of oestrogen levels are evident and wet days are appreciated. Ultimately an oestrogen level is sufficient to lead to maturation and ovulation with a Peak day takes place. It is important that these events are explained by the teacher.

I have had access to only a small number of cervical samples obtained during breast-feeding. However, it seems remarkable that there is an unexpectedly large amount of P mucus during the regress of an episode of follicle growth, not leading to maturation. This question has to be further investigated in the future.

Diseases and the Billings Ovulation Method

This is a very broad subject and I can only give some examples. Diseases are general or specific.

General Diseases

Anaemias. Anaemias are usually accompanied by an increased amount of L and S mucus. After treatment, these symptoms cease.

Asthma. This illness does not alter the secretions, but treatments in which adrenaline-like remedies are used elicits an increase in the lubricative sensation.

Jaundice. Jaundice is accompanied by liver malfunction, including metabolism of steroid hormones. Irregularities in menstruation and mucus secretion are common.

Specific Diseases

Inflammation of the Cervix and Vagina. These diseases are accompanied by an increased discharge and alterations in the quality of the flow. Fertile and infertile phases are sometimes indistinguishable. After treatment this imprecision is

improved.

The Pill. Complications arising from the use of the Pill are very frequent. Infertility after its use for 7-15 years is a very serious problem. S crypts are very sensitive to normal and cyclical stimulation by natural oestrogens and the Pill causes atrophy of these crypts. Fertility is impaired since the movement of sperm cells up the canal is reduced. Treatment is difficult. In some cases hormonal treatment is possible. In very intractable cases I have tried to reconstruct the S crypts microsurgically with acceptable results but the treatment is difficult and time-consuming. As already mentioned, a careful study of unsuccessful micro-operations led to the discovery of the P crypts and the P mucus.

The Future

I think that the principal secretions of the cervix have now been identified and characterized. However, several discoveries are expected, especially the enzymes produced in the isthmus of the cervix. Another important field is the cellular mechanisms involved in the differentiation and proliferation of mucus cells in the fetal cervix. The processes of reabsorption of vaginal fluids will constitute a subject for further investigation at the level of molecular biology.

We know that the Billings Ovulation Method is being used by probably more than 50 million women and families around the world, but we also know that several women have had difficulty in applying it. Today, young women are under increasing pressure to take the Pill and other hormonal contraceptives (injectables, progesterone-loaded IUD's or implants). In order to respond to these pressures it is necessary to have the knowledge and an understanding of cervico-vaginal physiology of the adolescent. In this way we are able to take care of young women in a manner that they are not deceived and so avoid using the Billings Ovulation Method but will overcome the difficulties and use that method for the rest of their lives. This is also important for the propagation of the Ovulation Method to the next generation of women and families.

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Author's note to Internet version of the paper

The morphologic classification of P mucus subtypes is P6 and P2. The used notation Pa refers to the mucolytic activity usually carried by the morphologic subtype P2.

The following corrections to the originally published version of the paper have been made.

In Figure 5: The scale divisions on the abscissa 1.0, 1,5, ... 3.0 have been corrected to 1.5,2.0, ..., 3.5.

In Table 2, the last row: The Mucolysis times for mucus types P6 and Pa of 15 hours have been corrected to 1.5 hours.

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Appendix

Old and New Names for Some Embryological Structures

Old Name	*New Name*
Urogenital fold	Urogenital ridge
Wolffian duct (also called Gärtner's duct)	Mesonephric duct
Miillerian duct	Paramesonephric duct
Pronephros	Same
Mesonephros	Same
Metanephros	Same
Gubernaculum	Same or Caudal genital ligament
Urogenital sinus	Same
	Inducer substance (if male characters are considered)
Miillerian inhibition substance (MIS) or Jost factor	
	Suppressor substance (if female characters are considered

Recommended literature:

"Medical Embryology", 4th Edition, 1981, by J. Langman. [Williams & Wilkins: London.]