

The points of time important in NFP therefore seem to be closely correlated to the secretion of the different types of cervical secretion.

Table I  
The subdivision of the typical ovarian cycle.

Phase	Days in the menstrual cycle	Days related to the probable day of ovulation	Dominating type of cervical secretion
Menstrual	1 to 5	-13 to -9	(G)
Post-menstrual	6 to 8	-8 to -6	G
Preovulatory	9 to 12	-5 to -2	L
Ovulatory	13 to 14	-1 to 0	S
(Peri-ovulatory)	12 to 15	-2 to 1	S
Post-ovulatory	15 to 28	1 to 14	G

It  
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TABLE II

Item	Vaginal	Cervical		
		S	L	G
Percent (ovul.)	-	22	75	3
Shape of units	-	strings	loafs	irregular
Size of units	-	50 $\mu\text{m}$ x 100 $\mu\text{m}$ x 20 mm	0.3 x 1 x 3 mm	0.5 x 1 x 2 mm
pH	4.5 - 5	7 - 7.6	7 - 7.7	6.5 - 7.2
Contents (percent)				
Mucin	1	0.9	1.3	4.5
Alb. + glob.	2	0.2	1.0	3
NaCl	0.6	0.8	0.8	0.6
Mn <sup>++</sup>	0.01	0	0	0
Micelles				
Diam. $\mu\text{m}$		0.4	0.2	0.04
Ordering		parallel	curved	isotropic
Intermicellar spaces				
Width $\mu\text{m}$		4	1.5	0.3
Ordering		parallel	curved	irregular
Leucocytes		very few	few	many
Liquid state	sol and cell suspension	gel	gel	gel
Microvisc. cp	4	2	5	15
Macrovisc. cp	10	100	1,000	10,000
Crystals		needles	palm leafs	small, irreg.
Stim. by horm.	estr. + prog.	high and late estr.	estr.	prog.
Neural stimulation		catecholamines?		acetylcholine?
Biol. role in fertility	direct sperm towards cerv.	rapid sperm swimming	capturing mal-formed sperm	closing cerv. after ovul.

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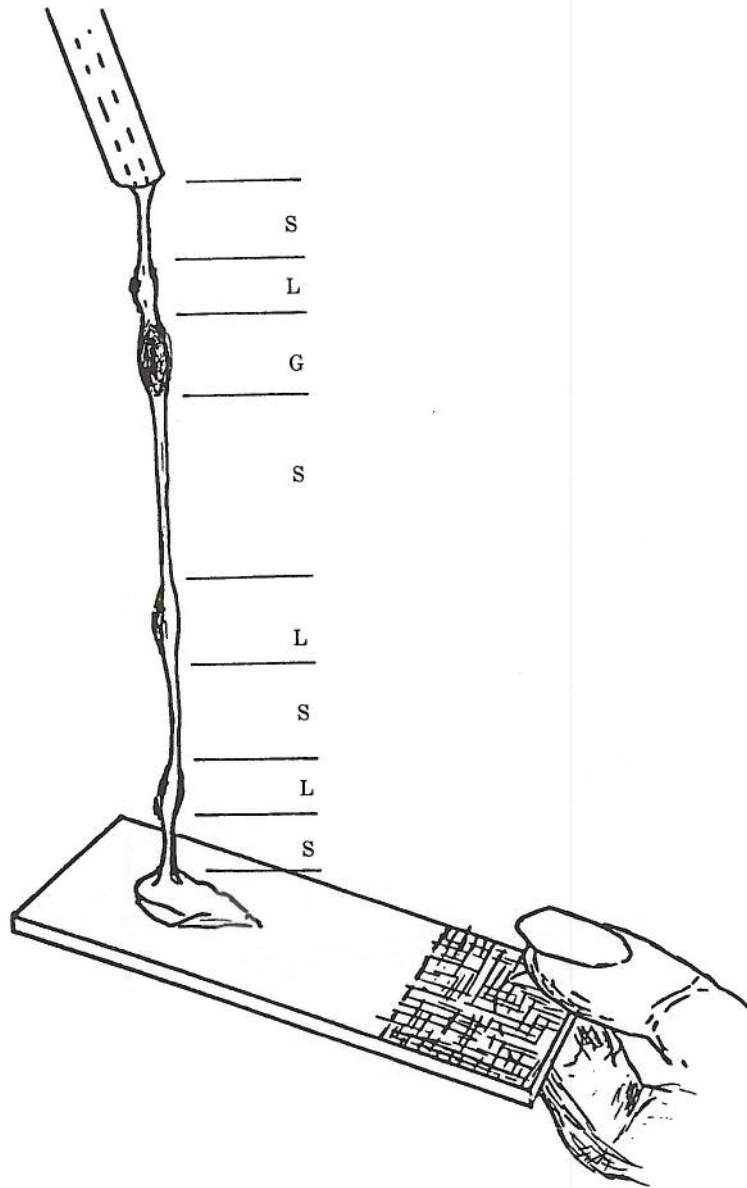
Dominating type of cervical secretion
(G)
G
L
S
S
G

TABLE III

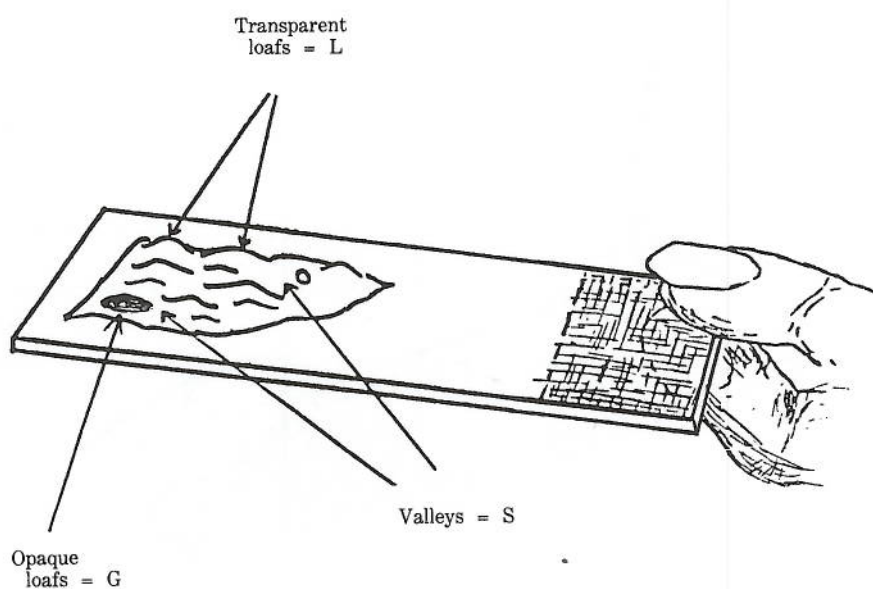
Woman's dominating observation of mucus	Deep internal sensation	No sensation at all	Sensation from lower vagina	Sensation or found in vulva	Total
Cervix only	12	9	0	0	21
Fornices or middle vagina	6	10	0	0	16
Lower vagina or paraurethral pockets	0	0	18	11	29
Vulva	0	0	7	40	47
Total	18	19	25	51	113

Fig. 1. W  
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ensation r found n vulva	Total
0	21
0	16
11	29
40	47
51	113



**Fig. 1.** When performing the spinnbarkeit test, one can see that the mucus thread is uneven due to the presence of different types of cervical secretion. The S and L types are transparent, the G type is opalescent. The thin parts of the thread denote the S mucus.



**Fig. 2.** When cervical mucus is smeared on a slide, the layer becomes uneven. The "hills" are L mucus if transparent, G mucus if opalescent. The "valleys" contain S mucus. The inspection must be done quickly, before the mucus dries.



**Fig. 3.** After microscope. Formations, or branched of an eye-pic

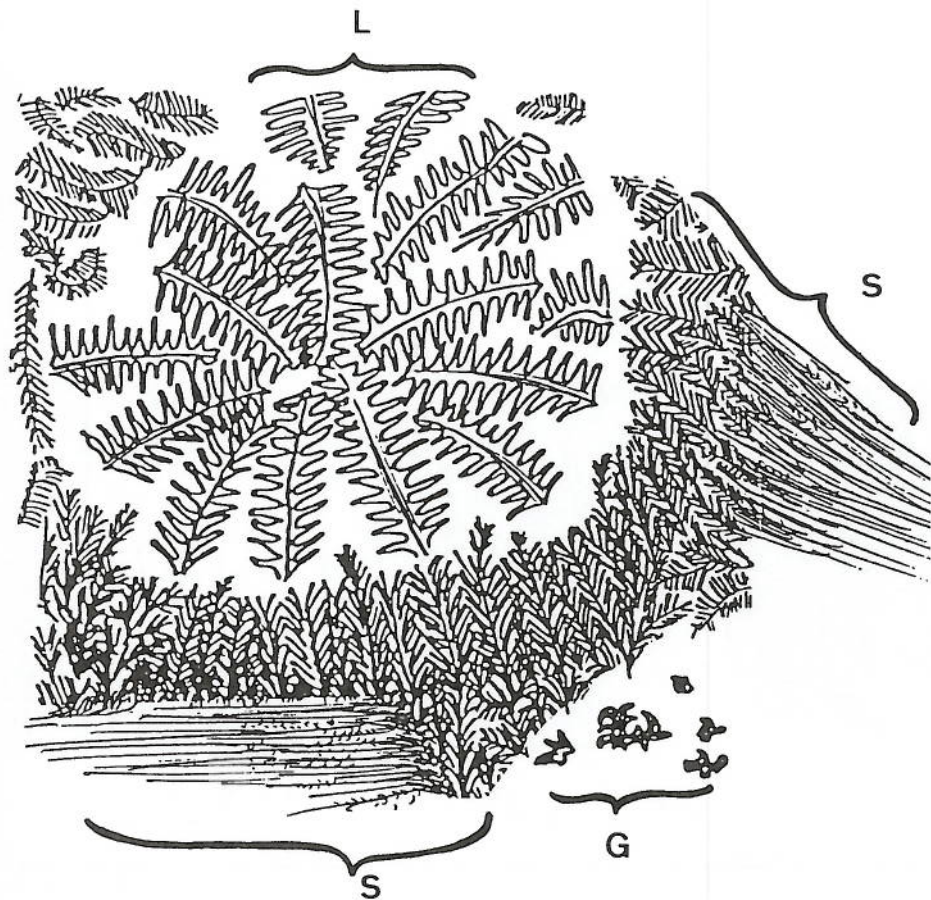


Fig. 3. After drying, three types of crystals can be identified at low magnification in the microscope. G type shows no or small irregular or cubic crystals. L mucus shows large palm-leaf formations, often concentrically arranged. S mucus shows thin crystals, both long single needles or branched needles. The areas covered by the different crystal types can be counted with the aid of an eye-piece square net.

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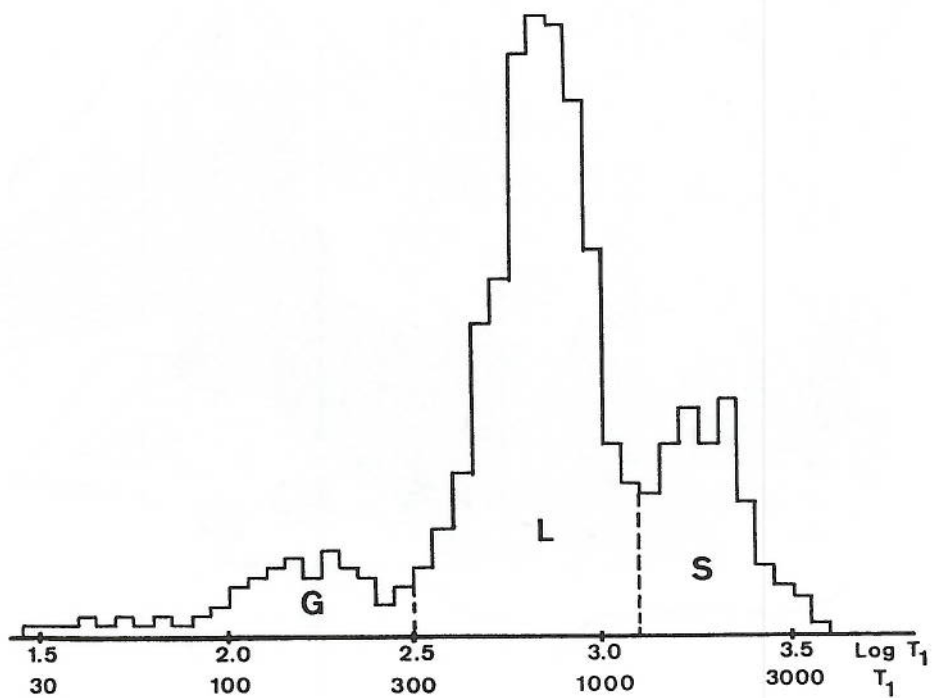


Fig. 4. Distribution of single-crypt mucus samples. Abscissa,  $\log T_1$  expressed in milliseconds. The S, L, and G types of mucus are clearly visible.



Fig. 5. Mucus combined by micelles, arranged in a complex, branching structure which are difficult to mix up and interpenetrate.

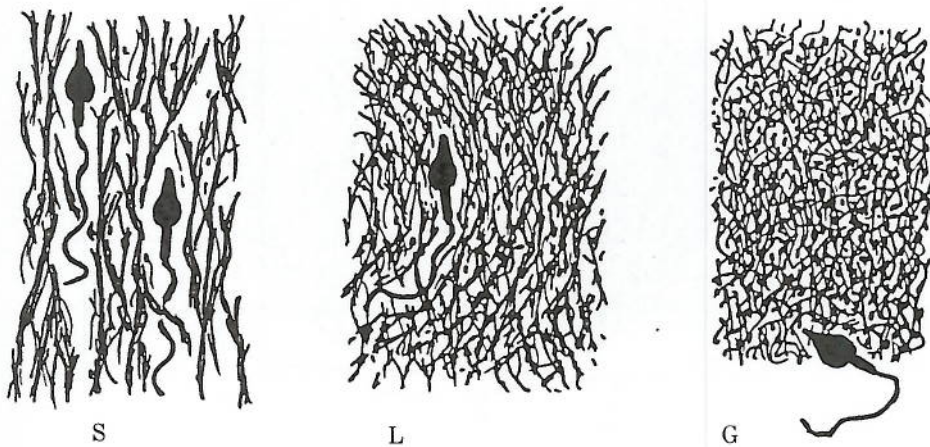
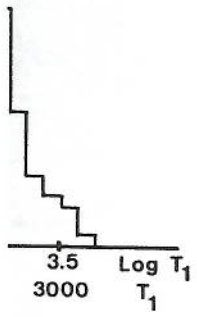
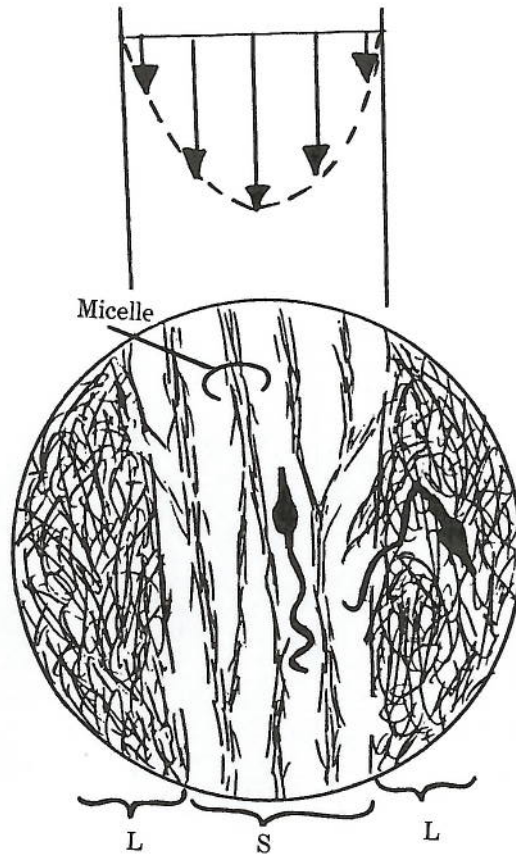


Fig. 5. Macromolecular mucin architecture in the three types of cervical mucus, resulting from combined biophysical studies with all methods listed in text. In S mucus the mucin molecules are arranged in parallel by flow orientation and interact to form micelles. The free spaces between micelles are 3 - 6 micrometers, permitting rapid sperm swimming in the intermicellar spaces, which are filled with low-viscosity water. In L mucus the structure is denser and sperm have great difficulty moving; average free space is about 1.5 micrometers. In G mucus the micelles are "split up" and intermicellar spaces are only a fraction of a micrometer; sperm cannot penetrate.

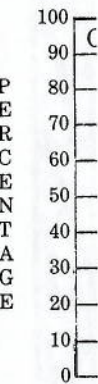


sed in milliseconds.



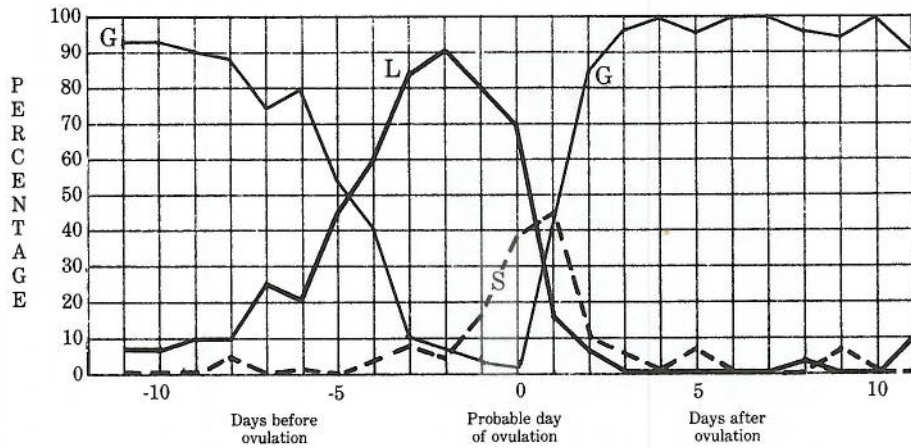


**Fig. 6.** This picture indicates a string of S mucus, the flow of secretion being indicated by arrows. This flow orients the mucin molecules when they happen to elongate by thermal motion phenomena. Then they interact and form micelles. One sperm is indicated to swim upwards. Another sperm, morphologically defective by angulation, is liable to deviate towards the borderlines of the S string, and finally it enters the E-L mucus and becomes captured in a loaf of L mucus. For more details on micelle structure and its hydration, see figure 17.



**Fig. 7.** The immediate increase until decrease in luteum phase.

The day after the external release from the whole upper exposing th



**Fig. 7.** The cyclic variation of percentage of G, L, and S mucus based on 1,124 cervical samples. Immediately after menstruation the G type dominates. When the estrogen stimulus on the cervical mucosa increases, the mucosa responds with increased L secretion. The S mucus does not increase until 1-2 days before ovulation and is actually high also the day after ovulation when it then decreases suddenly. The L mucus decreases about one day before the S mucus. During the corpus luteum phase, the G mucus dominates.

The day after ovulation, the G mucus is secreted from crypts in the lowest part of the cervix and the external os. This aids to close the cervical canal at its lower end. Above this "closed door" there is a very loose or liquid mucus "plug" consisting mainly of S mucus and containing the sperm released from the crypts which have been colonized during the first phase of sperm advance. The whole upper part of the cervical canal now acts as a big sperm reservoir, capable of continuously exposing the ovum to sperm.

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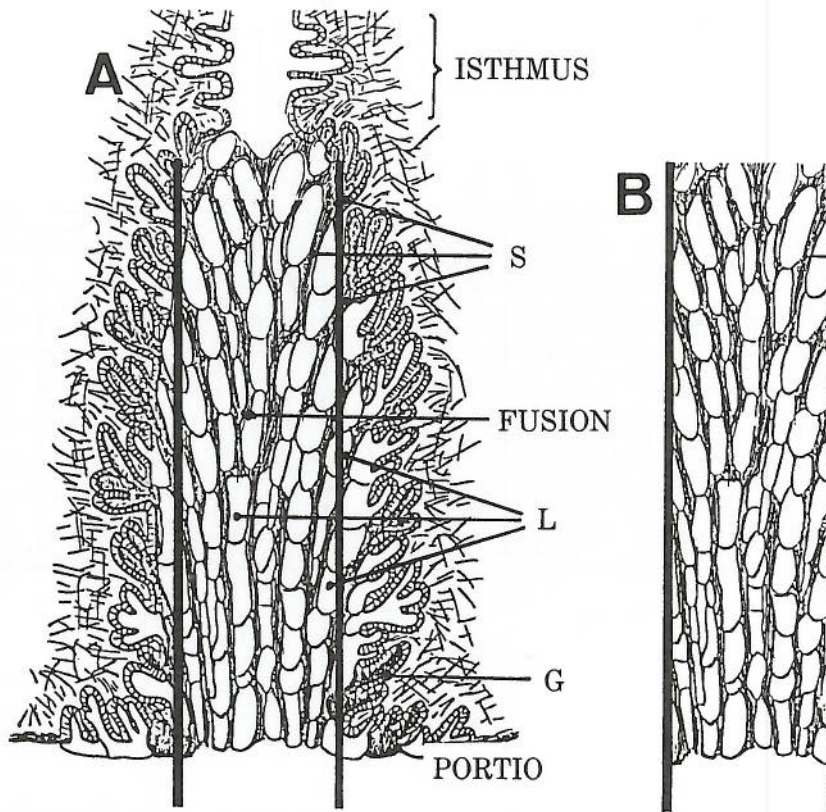
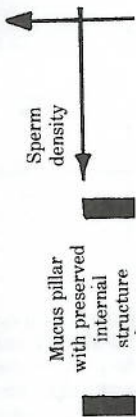
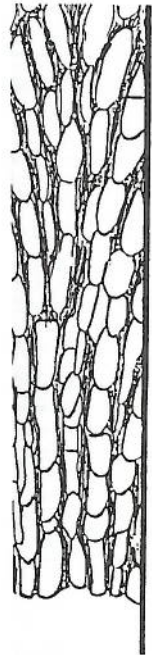


Fig. 8. Illustrating a technique to obtain cervical mucus with its internal structure preserved. A thin-walled glass tube is gently inserted into the cervical canal up to the isthmus region, with simultaneous application of a soft suction.

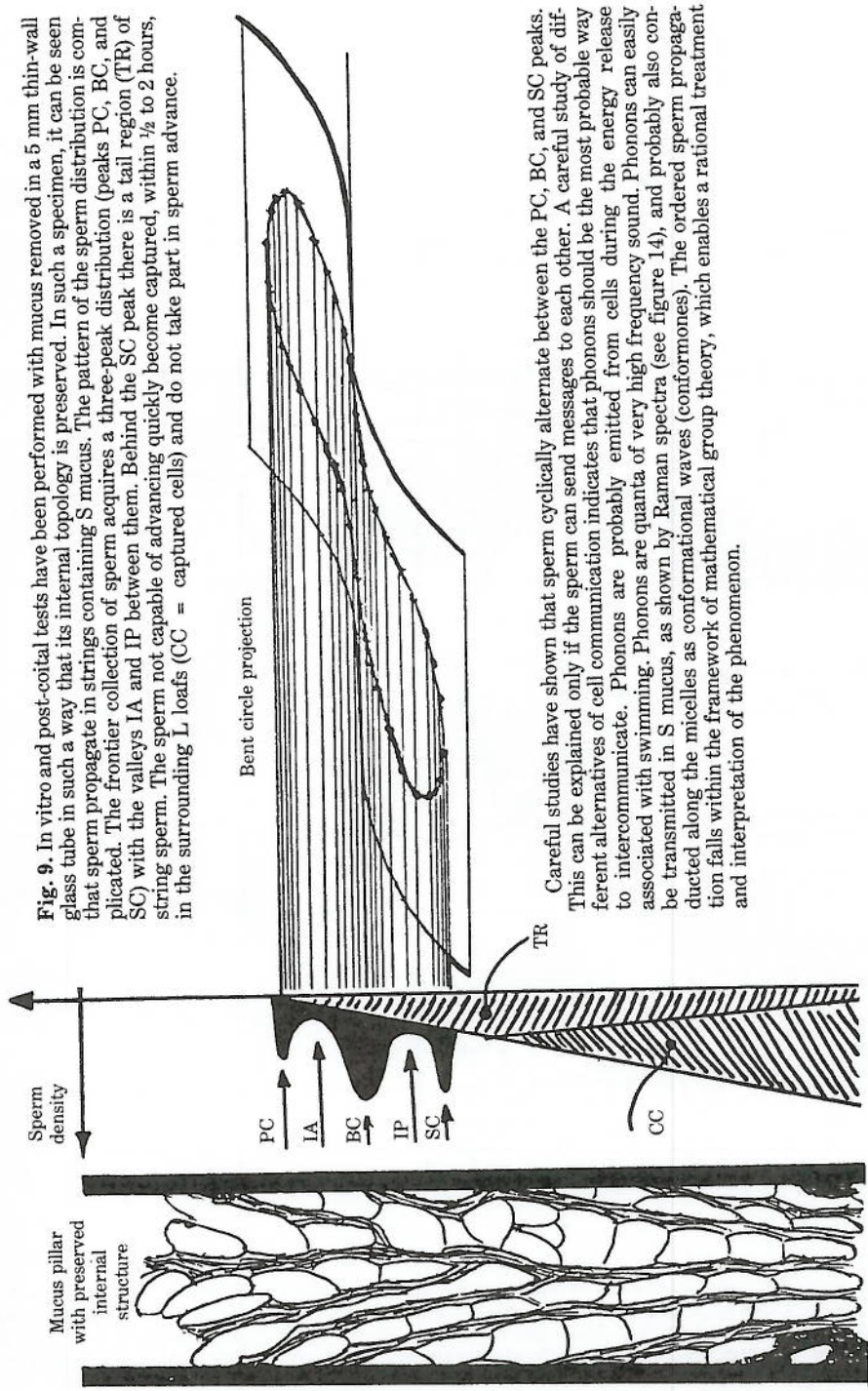
- A = Tube fully inserted
- B = Tube removed with preserved sample

Fig. 9. In vitro and post-coital tests have been performed with mucus removed in a 5 mm thin-walled glass tube in such a way that its internal topology is preserved. In such a specimen, it can be seen that sperm propagate in strings containing S mucus. The pattern of the sperm distribution is complicated. The frontier collection of sperm acquires a three-peak distribution (peaks PC, BC, and TR). Behind the SC peak there is a tail region (TR) of





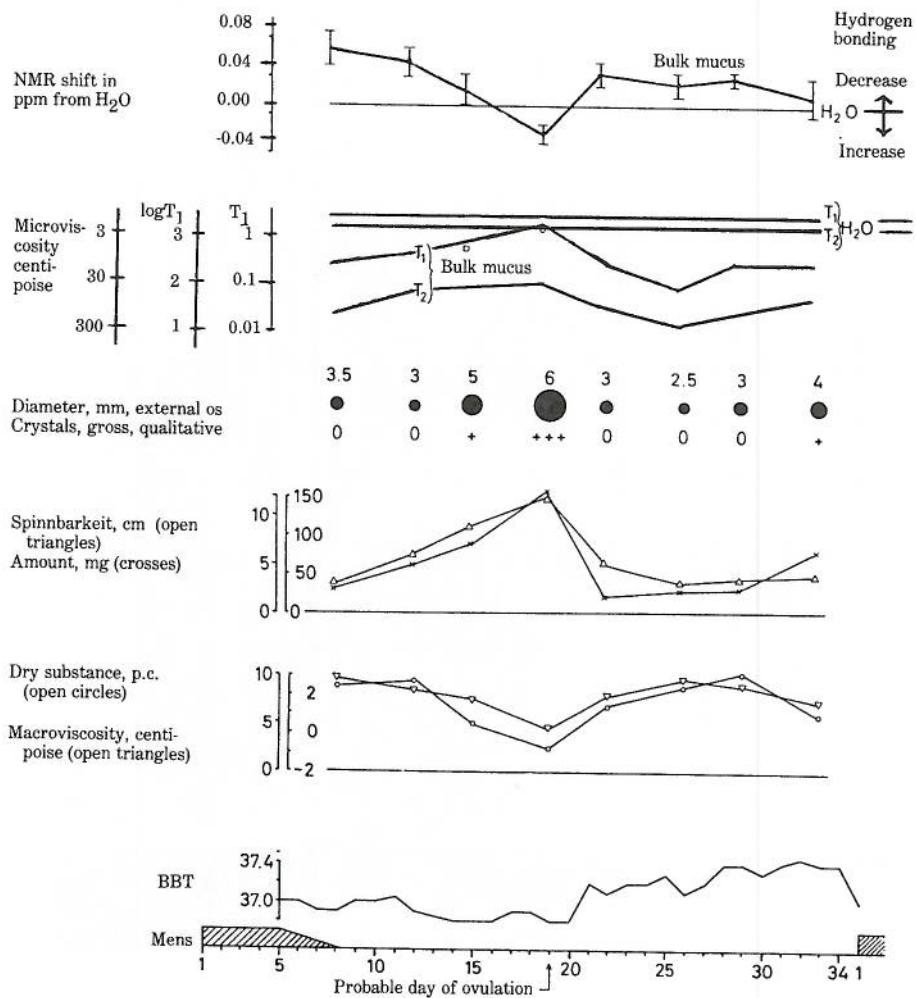
structure preserved. A  
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**Fig. 9.** In vitro and post-coital tests have been performed with mucus removed in a 5 mm thin-wall glass tube in such a way that its internal topology is preserved. In such a specimen, it can be seen that sperm propagate in strings containing S mucus. The pattern of the sperm distribution is complicated. The frontier collection of sperm acquires a three-peak distribution (peaks PC, BC, and SC) with the valleys IA and IP between them. Behind the SC peak there is a tail region (TR) of string sperm. The sperm not capable of advancing quickly become captured, within  $\frac{1}{2}$  to 2 hours, in the surrounding L loafs (CC = captured cells) and do not take part in sperm advance.

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Careful studies have shown that sperm cyclically alternate between the PC, BC, and SC peaks. This can be explained only if the sperm can send messages to each other. A careful study of different alternatives of cell communication indicates that phonons should be the most probable way to intercommunicate. Phonons are probably emitted from cells during the energy release associated with swimming. Phonons are quanta of very high frequency sound. Phonons can easily be transmitted in S mucus, as shown by Raman spectra (see figure 14), and probably also conducted along the micelles as conformational waves (conformones). The ordered sperm propagation falls within the framework of mathematical group theory, which enables a rational treatment and interpretation of the phenomenon.



**Fig. 10.** Cyclic variation of some cervical parameters in a healthy subject with a regularly long cycle (34 days). The upper curves show some NMR parameters in bulk mucus (chemical shift and relaxation times  $T_1$  and  $T_2$ ). Note the negative shift at ovulatory time indicating increased hydrogen bonding in S mucus, probably causing the very low viscosity and rapid sperm migration. Other parameters are evident from the picture.



**Fig. 11.** P changed w overwhelm peaks amo.

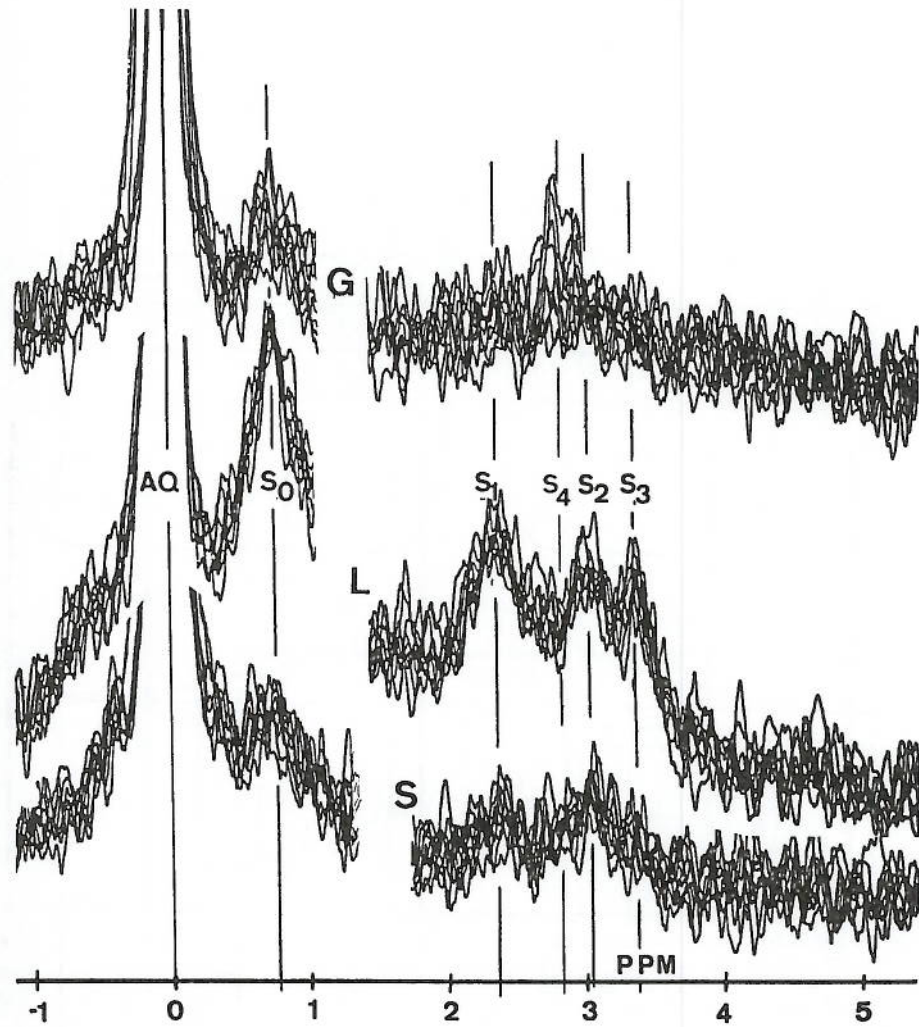
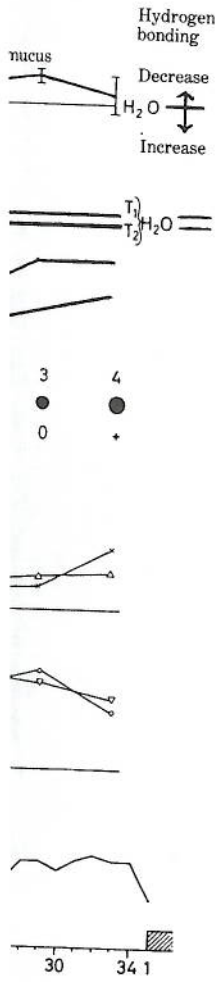


Fig. 11. Proton NMR spectra of cervical mucin of S, L, and G mucus. The water has been exchanged with 99 percent  $D_2O$  in order to depress the water signal (AQ) which would otherwise overwhelm the mucin peaks  $S_0$ ,  $S_1$ ,  $S_2$ ,  $S_3$ , and  $S_4$ . There are pronounced differences in these peaks among the three types of mucus indicating qualitative differences among the mucins.

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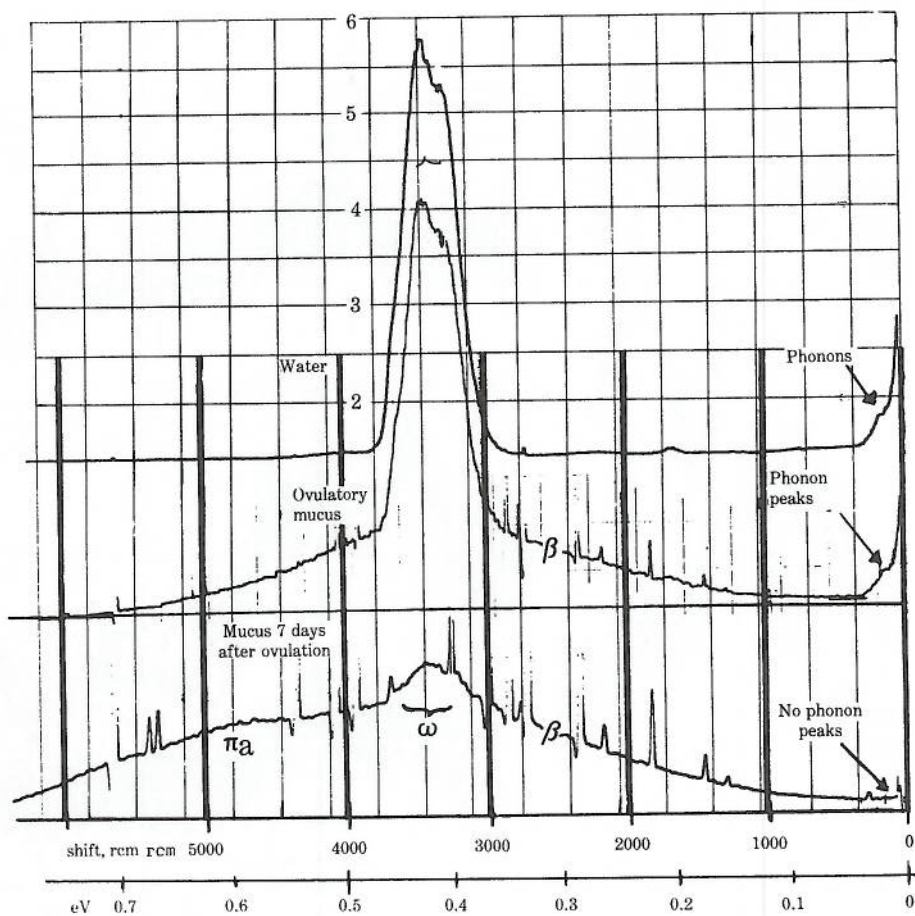


Fig. 12. Raman spectra of cervical mucus (bulk) from ovulatory and post-ovulatory periods. The four  $\omega$  peaks are seen in both, most pronounced in the ovulatory sample. A broad  $\beta$  peak is also seen, most pronounced in the postovulatory sample (G mucus). Also a peak denoted  $\pi_a$  is seen here. Phonon peaks ( $\phi$  peaks) are found only in the ovulatory mucus (S + L). The broad  $\beta$  bands contain contributions both from water and mucin.

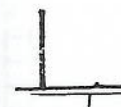
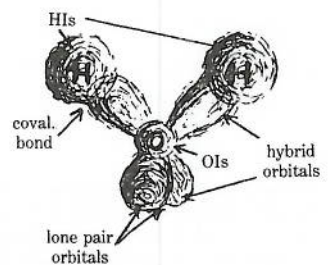
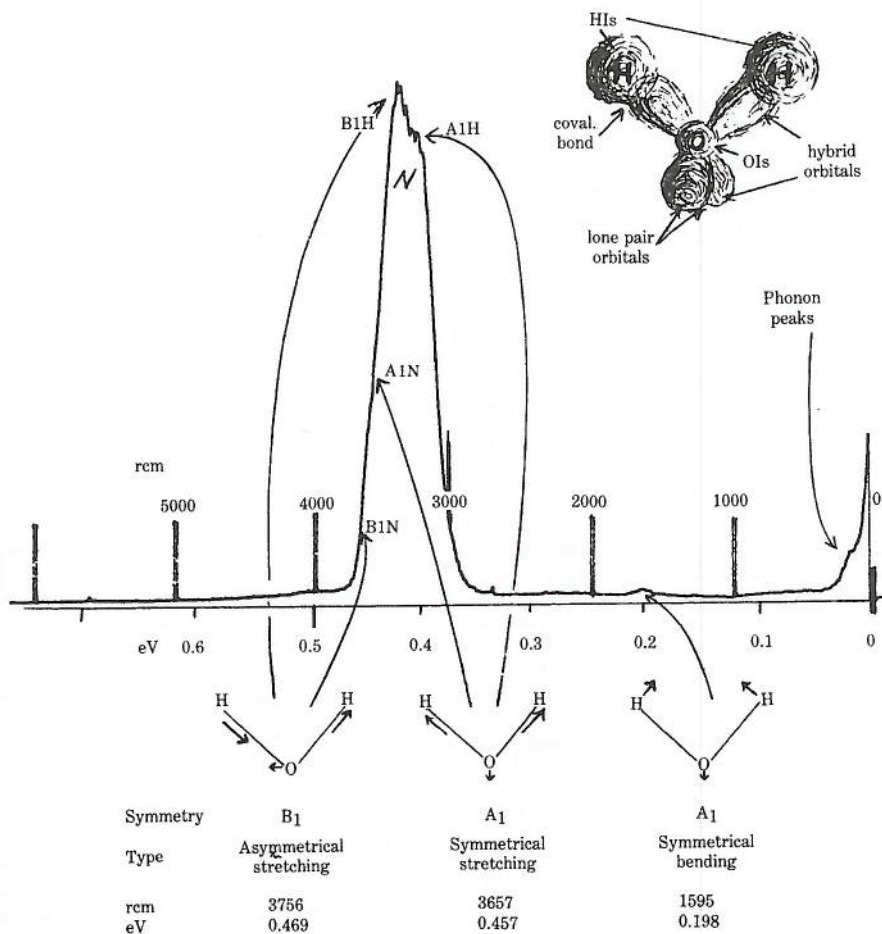
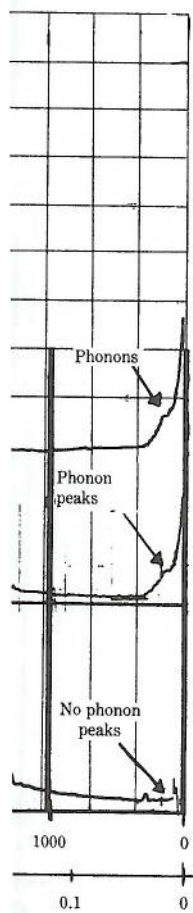


Fig. 13. The three-dimensional hydrogen bond structure (symmetrical hydrogen bond) is shown. The weak line at the four lines of computer. The phonon longitudinal shifts are in biological systems respectively.

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**Fig. 13.** The Raman spectrum of pure water and the water molecule. The top picture (right) is a three-dimensional representation of the water molecule with its orbitals. The three pictures (bottom) are schematic illustrations of the types of vibrations in water. Both the asymmetrical and symmetrical stretching vibrations give rise to two Raman lines, two small for "free" or non-hydrogen bonded molecules and two large for hydrogen bonded molecules. The lines are located at 3620 (B<sub>1</sub>N), 3520 (A<sub>1</sub>N), 3450 (B<sub>1</sub>H), and 3420 (A<sub>1</sub>H). The symmetrical bending has only one weak line at 1650 cm. The Raman shifts given in the picture refer to gaseous water. Note that the four lines B<sub>1</sub>N, A<sub>1</sub>N, B<sub>1</sub>H, and A<sub>1</sub>H overlap and can be completely resolved only with the aid of computer programs.

The phonon peaks occur in the region 60-230 cm. There are two large peaks, LA (= longitudinal acoustic) at 160 and LO (= longitudinal optic) at 190 cm.

Shifts are indicated both in cm (wave number in reciprocal cm) and in eV (electron volts).

In biological spectra, the lines B<sub>1</sub>N, A<sub>1</sub>N, B<sub>1</sub>H, and A<sub>1</sub>H are called  $\omega_{B1N}$ ,  $\omega_{A1N}$ ,  $\omega_{B1H}$ , and  $\omega_{A1H}$  respectively. The phonon peaks LA and LO are denoted  $\phi_{LA}$  and  $\phi_{LO}$  respectively.

latory periods. The broad  $\beta$  peak is also denoted  $\pi_a$  is seen. The broad  $\beta$  bands



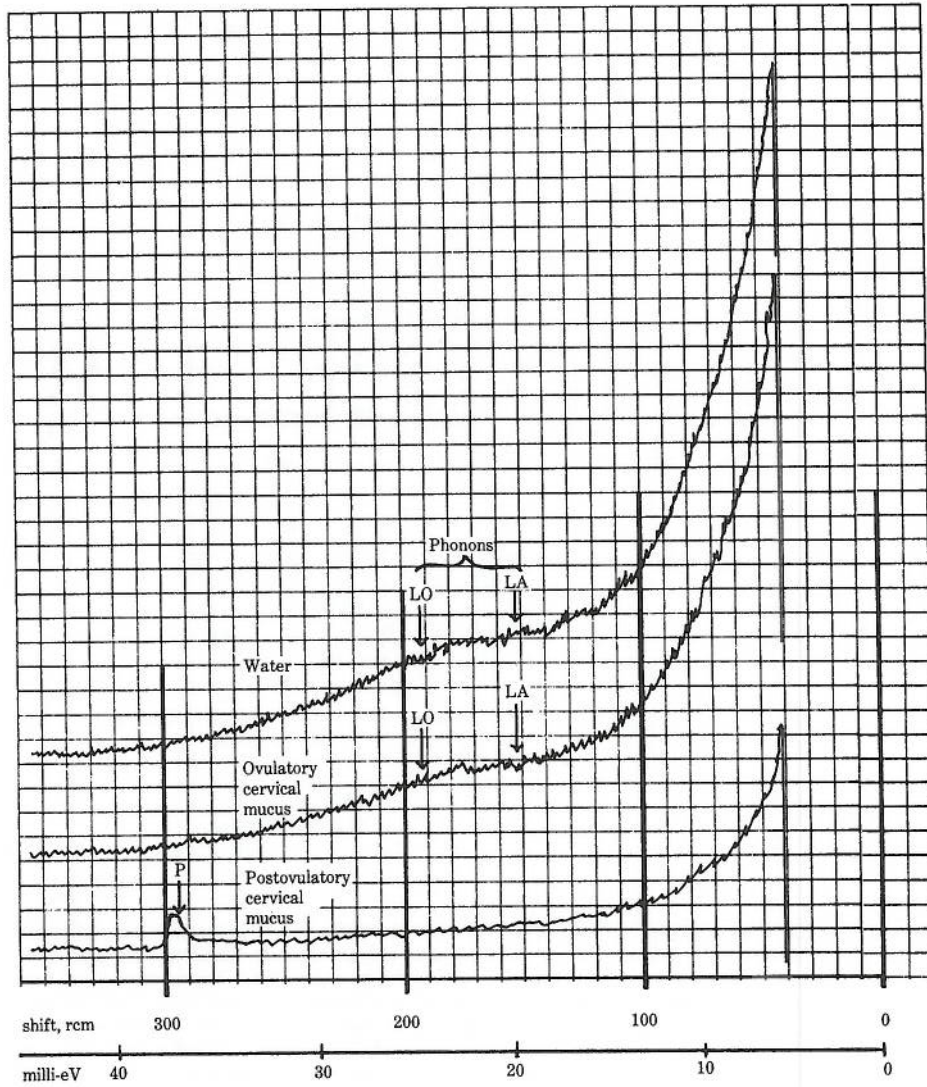


Fig. 14. Raman spectra of low shift of water, ovulatory and postovulatory mucus. G type mucus cannot carry phonons whereas water and ovulatory, mucus can.

Fig. 15. A pi pool, and vagi strings of S I semen pool. T toward the m

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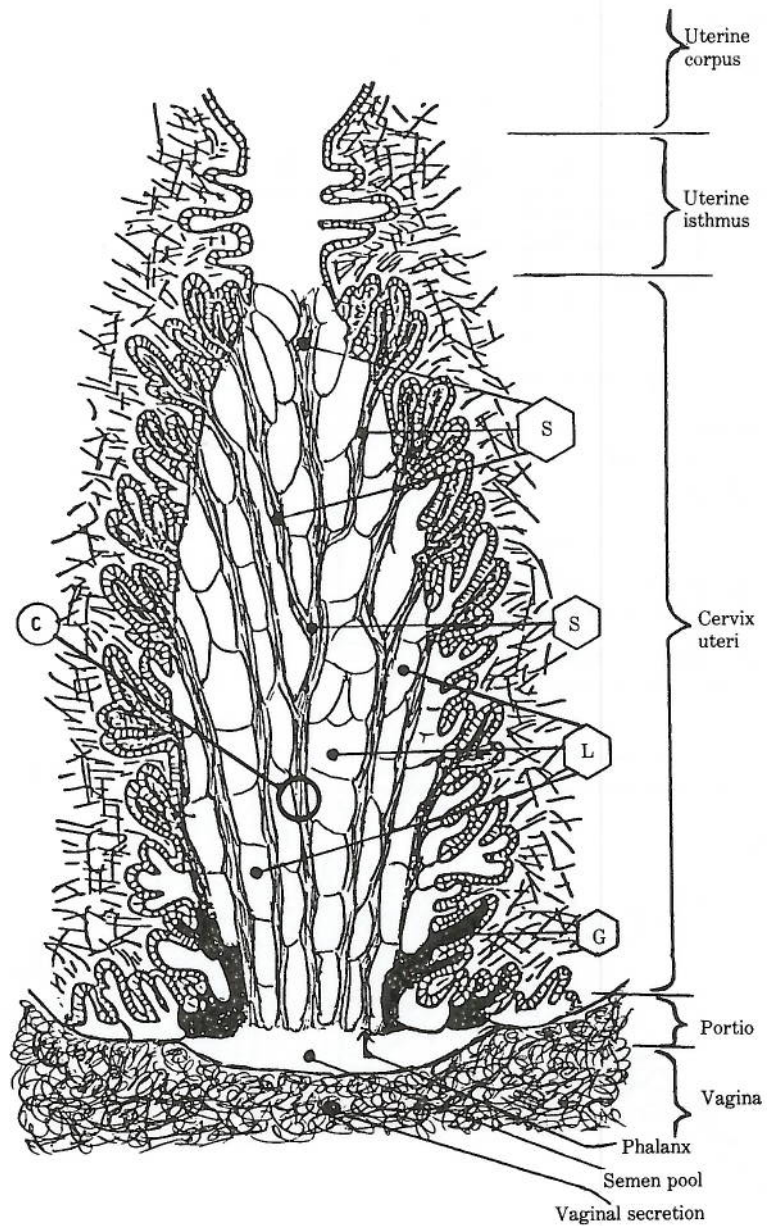
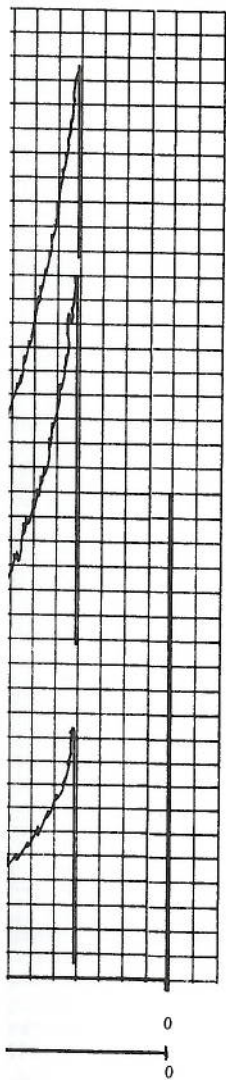
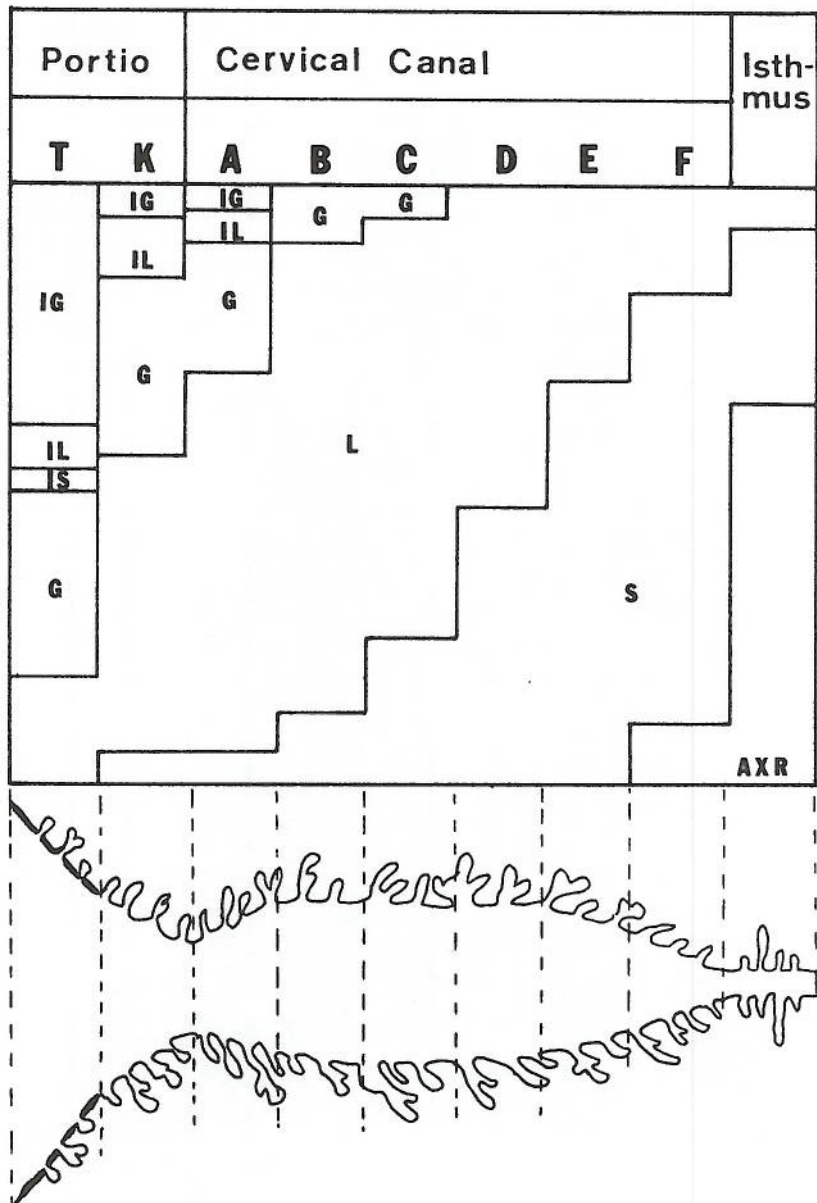


Fig. 15. A pictorial representation of the structure and topography of ovulatory mucus, semen pool, and vaginal secretions in situ. The ovulatory mucus is a mosaic made up of loafs of L mucus, strings of S mucus, and small pieces of G mucus. The S strings open as phalanges toward the semen pool. The acid vaginal secretion aids in generating a pH gradient orienting sperm upwards toward the mucus. The encircled area C is shown in higher magnification in figure 6.



**Fig. 16.** The distribution of different secreting units on the portio, in the cervical canal, and isthmus. The cervical canal is divided into six approximately equally long parts, A - F. The portio is divided into two zones, the regenerate or transformation zone (T) and the ectopic area (K) covered with "cervical" secretory cells. The anatomic layout below the diagram also helps to clarify the meaning of this illustration. The secretory crypts in the cervical canal are morphologically different from those in the isthmus. S, L, and G denote the corresponding secretory units. IS, IL, and IG denote "isocrypts" or "isoglands" (= noncycling units always secreting the same type of mucus). AXR denotes the serous secretion from the isthmus glands, which seems to contain a sperm-activating substance, tentatively called axreveillin.

**Fig. 17.** I tide back hydrated (to a sma

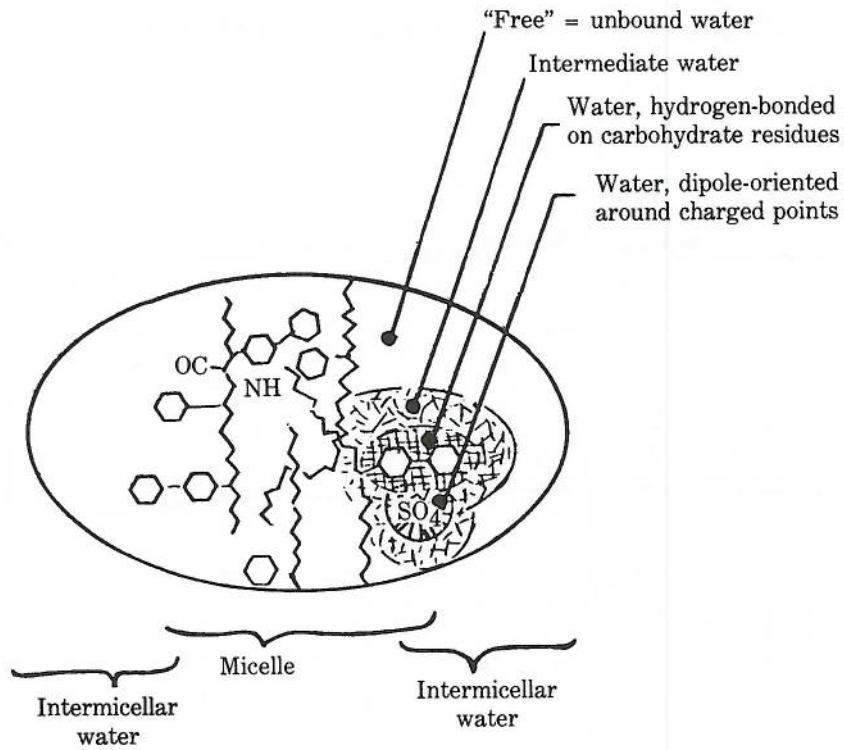
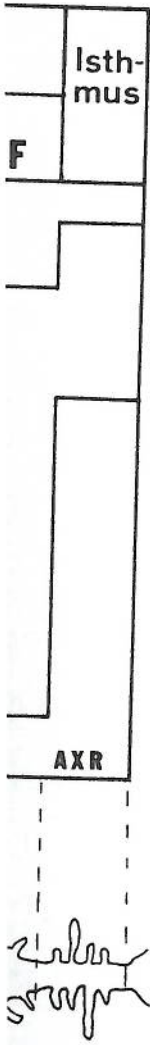


Fig. 17. Part of a micelle and surrounding water (encircled area of figure 6). Some mucin polypeptide backbones and attached sidegroups are indicated together with the free, intermediate, and hydrated water areas. Water is hydrated partly by hydrogen bonds, partly by dipole orientation (to a small extent). The net effect is increased hydrogen bonding.

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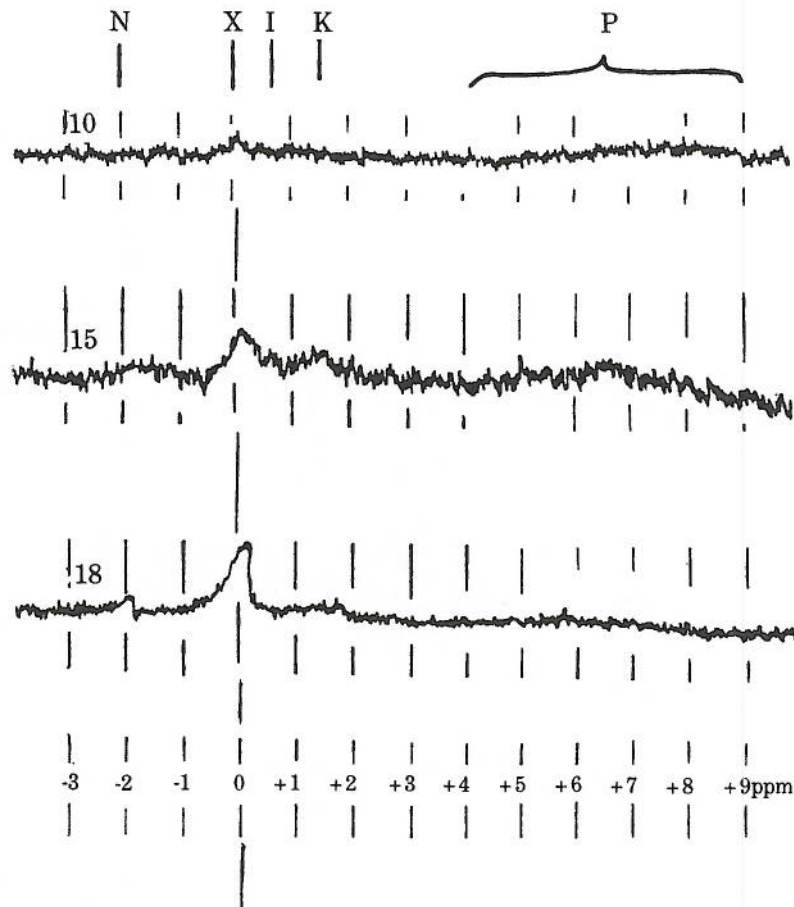


Fig. 18. Microproton NMR spectra of vaginal samples of preovulatory, ovulatory, and postovulatory mucus (water exchanged with 99 percent  $D_2O$ ). X is extracellular water, I and K are intracellular water peaks. N and P are protein bands. The barrier separating intra- and extracellular water is most pronounced in ovulatory samples.

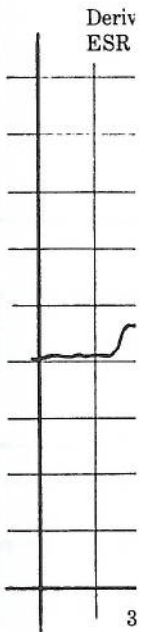


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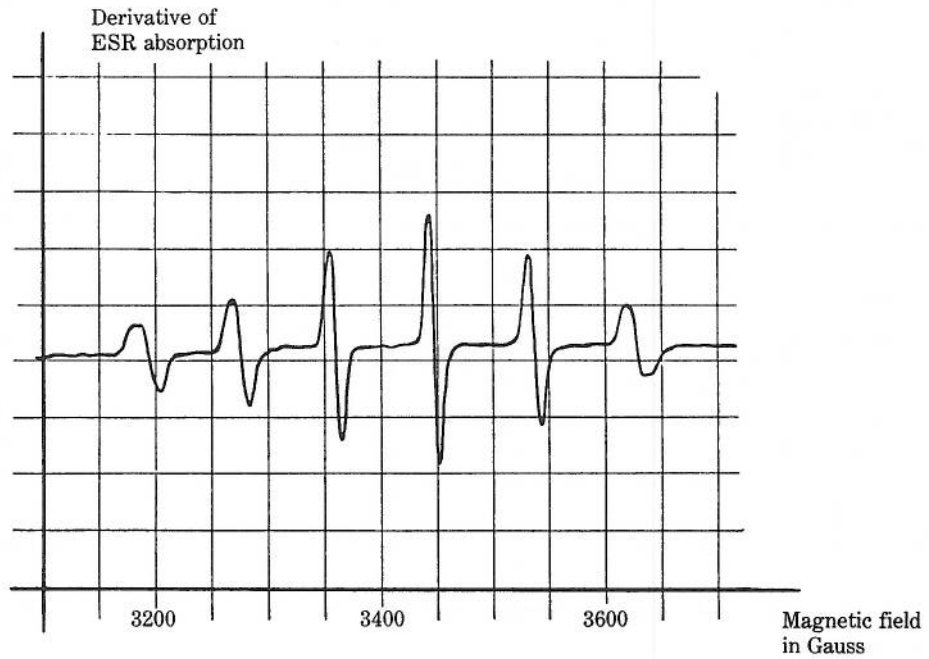
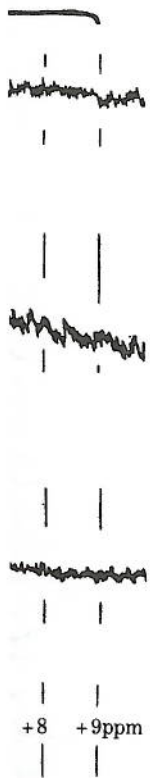
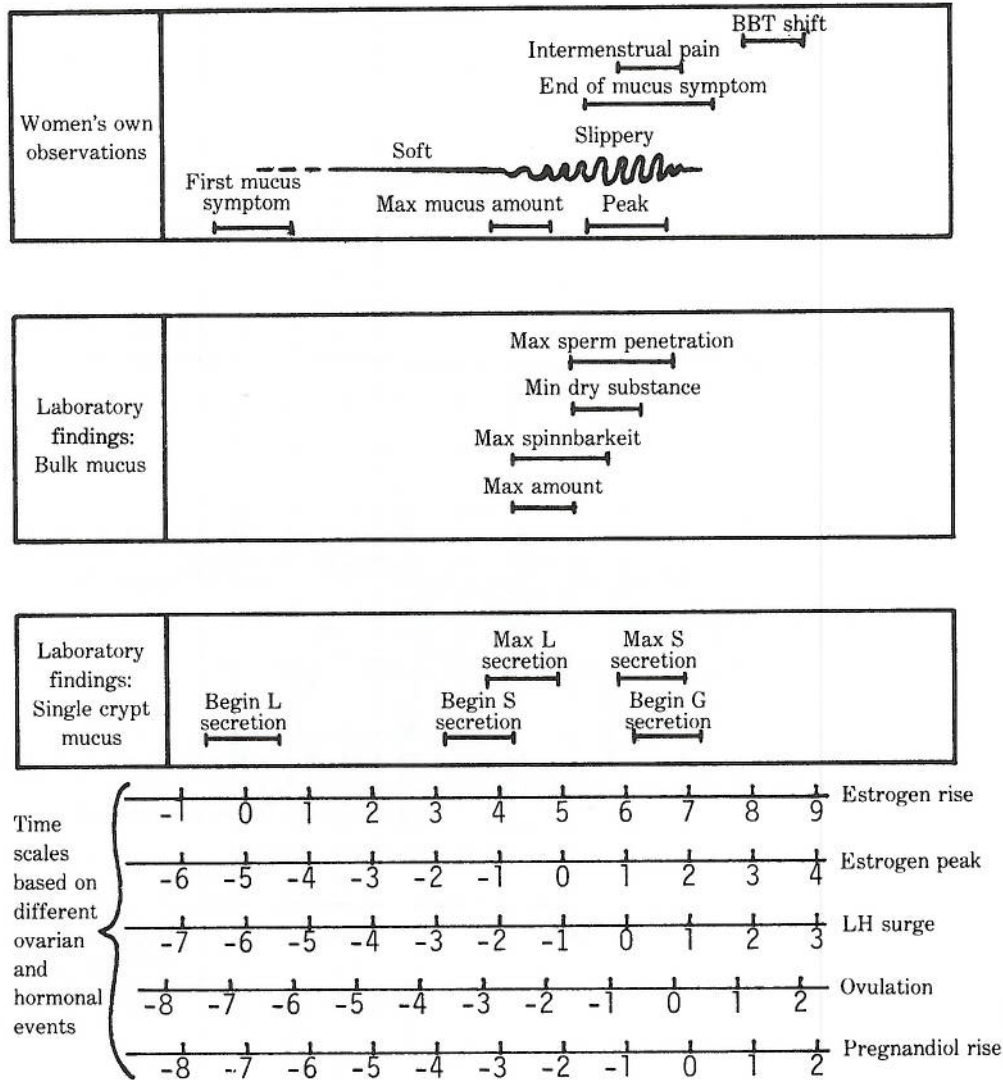


Fig. 19. This picture shows an ESR spectrum of dehydrated vaginal contents. The six spectral lines show conclusively the presence of manganese in the vaginal lumen. The cervical secretion contains no manganese at all, so it derives from the vagina. It amounts to about 0.2-1.0 millimolar concentration and seems to be present as free ions. Its role is not yet known. Some data indicate that manganese may compete with zinc in the seminal plasma and in this way be involved in fertility in a way not yet understood.

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**Fig. 20.** A schematic diagram showing the approximate relations between various hormonal events associated with ovulation and the symptoms and signs related to ovulation as well as some biophysical properties of cervical mucus. The data underlying the diagram are obtained from an attempt to weigh together data from the following papers: Bergman 1950; Hartman 1962; Moghissi et al. 1972; Billings, Billings, Brown, & Burger 1972; Flynn & Lynch 1976; Casey 1977; Vollman 1977; Hilgers, Abraham, & Cavanagh 1978; Matthews et al. 1980; Billings & Westmore 1980; Cortesi et al. 1981; Billings 1981; Burger 1981; the present work.



**Fig. 21.** A schematic diagram showing the approximate relations between various hormonal events associated with ovulation and the symptoms and signs related to ovulation as well as some biophysical properties of cervical mucus. The data underlying the diagram are obtained from an attempt to weigh together data from the following papers: Bergman 1950; Hartman 1962; Moghissi et al. 1972; Billings, Billings, Brown, & Burger 1972; Flynn & Lynch 1976; Casey 1977; Vollman 1977; Hilgers, Abraham, & Cavanagh 1978; Matthews et al. 1980; Billings & Westmore 1980; Cortesi et al. 1981; Billings 1981; Burger 1981; the present work.

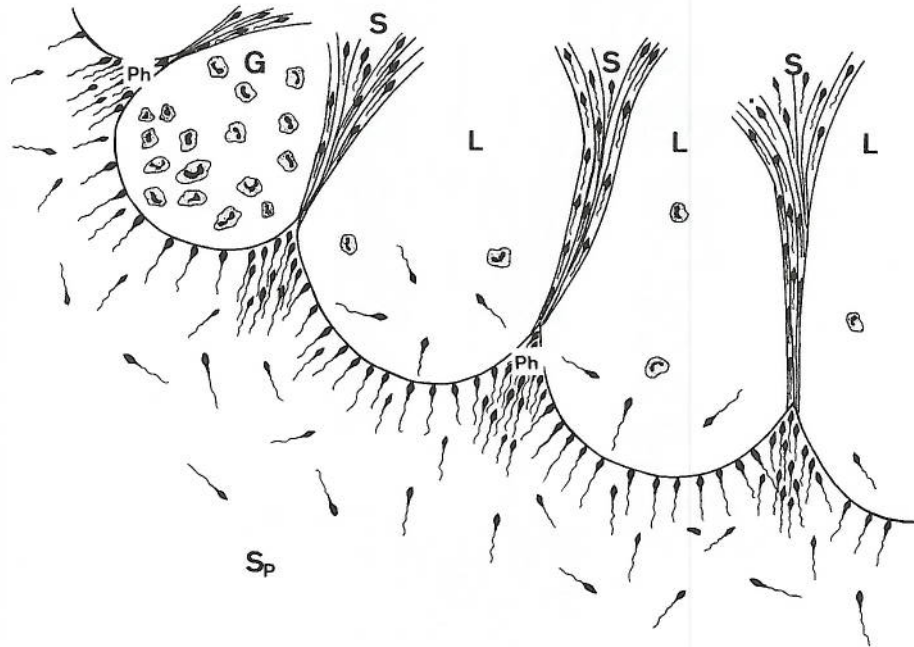
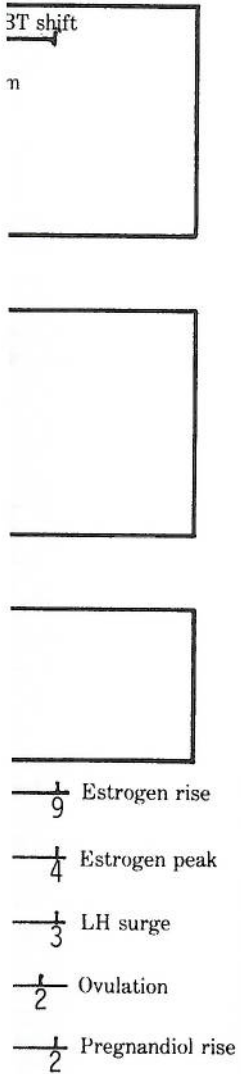


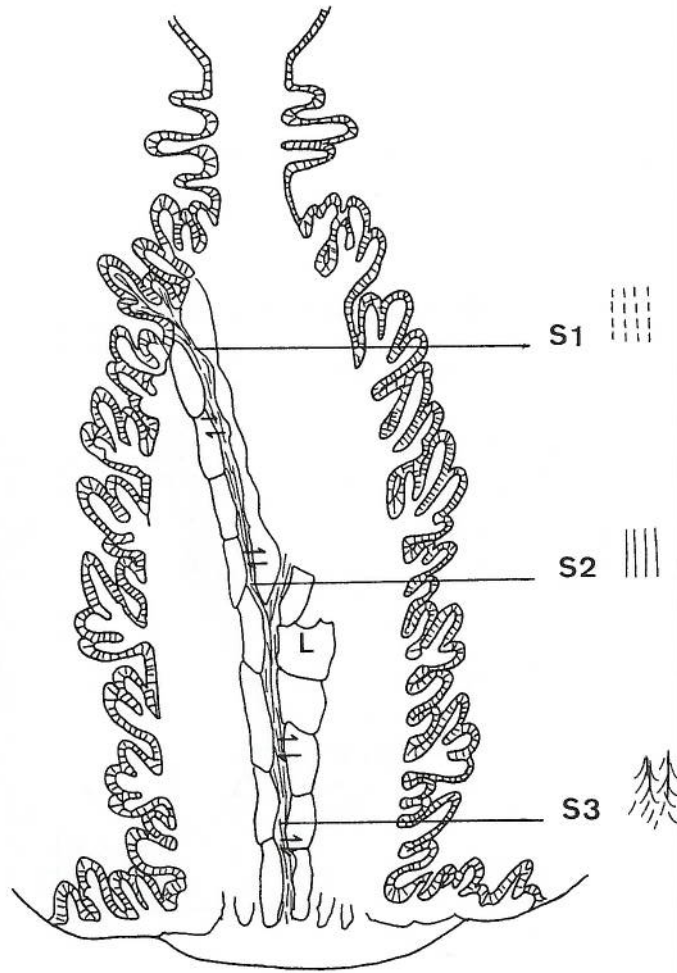
Fig. 21. A schematic illustration (not drawn to scale) of the formation and significance of phalanges in the slide test. According to our studies, the phalanges occur in the creeks which happen when the L mucus loafs are squeezed out between slide and cover. The S mucus is compressed between the loafs but may reach the edge in some points between the loafs. When sperm comes into contact with this highly deformed cervical mucus, the spermatozoa tend to accumulate in the creeks, and by thermal motion and/or sperm activity, the cells start to invade the S mucus. The string material is often deformed in such a way that the spermatozoa appear to fan out after traveling some distance.

Sp = sperm pool. Ph = phalanx. S = deformed string of S mucus. L = deformed loaf of L mucus. G = deformed unit of G mucus.

Some sperm are capable of invading directly into the L units and following irregular paths in the loaf. This corresponds to the "wide front" invasion of Bergman 1950. The sperm invading from a phalanx correspond to "caravans" of Bergman. The phalanx formation was described by Moghissi et al. 1964 and were further discussed by Moghissi 1973.

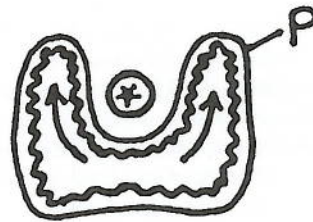
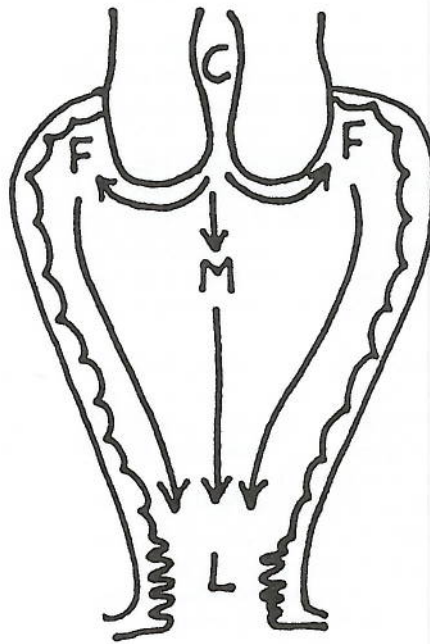
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**Fig. 22.** Illustration of how the S mucus may change continuously during the passage from the crypt to the external os. The S mucus flows between the "pebbles" or loafs (L). The passage may take 20 minutes to several hours, depending on the secretion rate and length of passage. During the flow, the S mucus exchanges molecules with the surrounding units of L mucus. The exchange of small ions like sodium and potassium ions is rapid due to rapid diffusion. Albumin and globulins exchange more slowly, and mucin molecules still more slowly as they are larger. Because L mucus contains more mucin molecules than the primary S mucus, the net diffusion effect is an increase of the mucin content in the string. This affects the crystallization pattern which changes continuously as shown in the small pictures to the right. According to the crystal shape, we can identify three variants of the S mucus, denoted S1, S2, and S3 with an increasing amount of mucin. If the flow of S material becomes very slow, or ceases completely, the string mucus loses its identity and becomes similar to the L mucus, which is more abundant in the cervical canal. As mentioned before, it is the flow of S mucus which tends to orient the mucin molecules in the string, so that the long molecular aggregates, called micelles, are formed. The spermatozoa normally ascend by swimming in the intermicellar fluid, which is very similar to saline plus low-molecular compounds such as amino acids, glucose, etc.

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**Fig. 23.** This figure depicts the flow of liquid material in the human vagina. The mucus coming from the cervix flows into the fornices, right, left, or posterior (F), more seldom to the anterior fornix. It may also flow to the middle part of the vagina. From these sites it may flow downwards to the lower part of the vaginal lumen (L) or to (P) the paraurethral pockets (Shaw pockets), where the mucus membrane often has a pronounced papillary surface. Reabsorption of liquid seems to take place here. The concentration of manganese ions in the extracellular vaginal fluid is highest here, and may amount to about 1 millimolar concentration. If mucus is not degraded before reaching the L or P regions, it can be sensed very well. Also, if the mucus reaches the vulva, it can be sensed or felt. This is evident from table III. As shown in this figure and discussed in the text, some women may also have deep sensibility for mucus in the cervical canal.

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