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Cervical Factors

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AS FAR as present knowledge extends, any sperm reaching the ovum has to pass through the contents of the cervical canal. The cervical-canal mucus therefore occupies a key position in human fertility. Because sperm transport, active, passive, or combined, is a biophysical phenomenon, the biophysical properties of the cervical mucus and their relations to sperm migration are extremely important. *Clift* (1946) has already expressed the opinion that there might be some relation between the molecular arrangement in the cervical secretion and sperm penetration. A detailed model of the molecular arrangement of the human cervical mucus was given by *Odeblad* (1959). A similar structure of bovine ovulatory mucus was also proposed by *Tampion and Gibbons* (1962). The cervical mucus is the end result of complicated biosynthetic processes occurring in the epithelial cells of the cervical mucosa. The biosynthesis is regulated by many factors. Steroids are known to be important regulators, but also other factors come into play.

The experimental investigations underlying this work have, as far as possible, been performed under such auspices that irrelevant contributions can be disregarded, so that only the cervical mucus proper has been investigated. In our department a battery of experimental methods of investigations have been used for studies of cervical mucus. Such methods are sperm-migration measurements, rheological studies, crystallization studies, cell countings, NMR, EPR, and photoelectron spectroscopy. These and other techniques have been described in a series of previous papers to which the reader is referred (*Odeblad* 1976). In the present paper the most important results and their significance for fertility problems will be discussed.

Dr. Erik Odeblad, of the Department of Medical Biophysics, University of Umea, Sweden, is internationally known for his use of modern techniques to study the physical and chemical properties of cervical mucus. His paper is reprinted from *Contributions to Gynecology and Obstetrics,* vol. 4, pp. 132-42 (1978), with permission of the publisher, Karger (Basel).

Earlier Studies on the Types of Secretion

In 1969 the two main types of secretions dominating the normal cervical sample were characterized (*Odeblad* 1969). These two types have been called type E and type G. The E secretion is characteristic for estrogenic stimuli on mucus biosynthesis, type G for gestagenic stimulation. The two types are always mixed with each other in different proportions. Their pathology is given in table 1. At an adequate estrogenic stimulus, for example during normal ovulatory phase, the E type dominates. The G type of secretion dominates during normal luteal phase or on adequate medication with an oral contraceptive of the combined type. However, they never occur in pure form but always side by side. Experimental investigations using NMR and other experimental techniques have shown that, at normal ovulation, there is about 97% of type E and 3% of type G. At normal corpus luteum phase there is about 10% of type E and 90% of type G.

As will be discussed later on, the very important finding has recently been made that the E type of mucus consists of two components, E_S, and E_L, both of which occur under estrogenic stimuli and are the results of slightly different biosynthetic procedures in different secretory units.

Besides the main types of cervical secretion, namely, E_S, E_L, and G, other forms have also been identified. The mucus occurring in so-named chronic cervicitis has been called type Q (*Odeblad* 1969). Type Q is not uniform and well defined but is composed of partial components depending on the type, degree, and duration of the inflammatory condition. The crypts releasing the type Q secretion are often relatively nonreactive to the endocrine stimuli. Such secretory units have been called isomucorrhoic glands, isoglands, or isounits (*Odeblad* 1966). Their biosynthesis is pathologic in the sense that their response to hormonal insults is inadequate.

The "nonreacting" structures producing a Q type of cervical secretion are therefore called iso-Q units. Iso-G and iso-E units are also common. They produce G or E types independent of the hormonal stimulus. Isounits also occur in acute inflammation and produce a serous type of secretion of low viscosity and high leukocyte contents, the V type. It must be pointed out that the secretion in most cases of cervical inflammation is therefore always a mixed secretion containing all the types, E_S, E_L, G, Q, and V, in different proportions varying from case to case and during the clinical course of the disease.

It was reported (*Odeblad* 1963) that tall, asthenic girls usually produce a secretion of a special kind. It is similar to, but differs from, the E mucus because it is exceptionally fluid, has high water content, and has a low water-binding capacity compared with the common E types.

In 1969 this cervical secretion was denoted the type H_1. A similar type could be isolated after prolonged, moderate treatment with estrogenic hormones (type H_2). If very intense medication with an estrogenic hormone is instituted there is, within a day, obtained a fluid secretion denoted H_3. Even if types H_1, H_2, and H_3 differ slightly, they have many properties in common, for example the large amount of mucus, the low contents of dry substance, and low water-binding capacity. The nature of the H type secretion from a biosynthetic point of view is not yet clear. Probably the types H_1, H_2, and H_3 are often mixed with each other and also very often mixed with the similar normal types E_L and E_S.

Several years ago it was found (*Odeblad and Rosenberg* 1968) that if the cervix is mechanically stimulated with a cotton swab, a transudate of serum proteins is released into the cervical canal. We called this cervical secretion type B. No biosynthesis in the cervical epithelium is involved in the formation of B type secretion. We have reasons to believe that, to some degree, transudation normally always occurs to the cervical mucus, forming part of the cervical plasma that is normally present in the aqueous phase between the gel-forming macromolecules in the cervical secretion. However, the E_S mucus seems to contain a very small amount of proteins.

In 1972, the action of retrosteroids on the cervical mucin biosynthesis was preliminarily reported. A characteristic opaque mucus, containing many cells, occurs. This mucus is called type R. It is present in large amounts and is not receptive for sperm invasion.

Molecular Architectures and Intermicellar Spaces

The E types of secretions have characteristic molecular architecture. The mucins contain macromolecules that are polypeptides with carbohydrate and other side groups attached at certain points of the polypeptide chain. The mucin macromolecules are elongated or thread-like. They occur in assemblies in which they lie more or less in parallel. The assembly as a whole is called a micelle. The micelles vary in diameter and arrangement. They are to some extent branched, and cross-linking between micelles occurs, which aids in the formation of the network gel structure. The E_S and E_L types both exhibit fairly ordered parallel arrangement of micelles with little branching and cross-linking. The spaces between the micelles are, however, of varying widths. In the E_S material most spaces are so wide that sperm may easily swim in the spaces. In the E_L material a considerable fraction of the spaces are so narrow that they do not permit sperm propagation.

The G type of mucus has thin micelles with many interconnections. The intermicellar spaces are irregular and narrow and do not permit entrance or progress of sperm. The R material is similar to the G type but has somewhat wider meshes of the micellar network.

The molecular-architecture parameters of the other types are less understood and are not discussed here.

It is evident from the preceding discussion that the intermicellar spaces play a fundamental role for sperm propagation. Besides the typing of the mucus materials and their molecular structures, the channels or spaces can also be classified. Three main classes have hitherto been characterized. They are: (1) DT = directed, sperm-transmitting spaces, 2-5 μm wide (E_S mucus and the H types); (2) DN = directed but not sperm-transmitting space, 1-2 μm wide (E_L mucus), and (3) NO = nonoriented spaces with no sperm-transmission capacity, spaces 0.3-1 μm wide (mucus types G and R).

The formation of micelles and thus the formation of intermicelle spaces is probably due to self-assembly mechanisms between the mucin molecules. The conformational properties of mucin are probably very important factors for the self-assembly processes, and conformation is, in turn, dependent on biosynthesis.

It must be stressed that molecular assemblies like micelles are not static formation but are very dynamic structures, vibrating and of varying shapes. Therefore, the intermicellar spaces also vary in size.

Recent Studies on the Architecture of Human "Midcycle" Intracervical Mucus

A significant advancement was made in 1976 when the E type, previously believed to be homogenous, could be resolved into two components called E_S and E_L (S stands for string, L for loaf). The two components are always present at the same time but have different topographical arrangement in the cervical canal, different properties, and different functions. The heterogenous nature of the E mucus was recognized as early as 1959 (*Odeblad* 1959, pp. 126-127), but at that time and during the intervening time period, no well-founded biological meaning was ascribed to this knowledge. A long-term study with micro-NMR analysis of single-crypt contents was compiled in the spring of 1976, and comparison with sperm migration on well-preserved mucus explants soon revealed the new findings.

The occurrence of two types of estrogen-type cervical secretions, as well as their biological function, was then established. The E_S mucus conveys the spermatozoa from the vaginal pool, but the E_L type is largely inactive in this respect.

Ovulatory mucus contains 20-25% type E_S, 72-77% type E_L, and 3% G. It is important to realize that the strings are the result of biosynthesis in certain functional units (crypts) in the cervical mucosa and that each loaf is also the result of a biosynthetic process in another-type functional units (as of course also the few percent of G mucus). It is also important to realize that new strings are formed, "old" strings disappear, and the E_S-E_L system is very dynamic. Figure 1 illustrates the situation schematically.

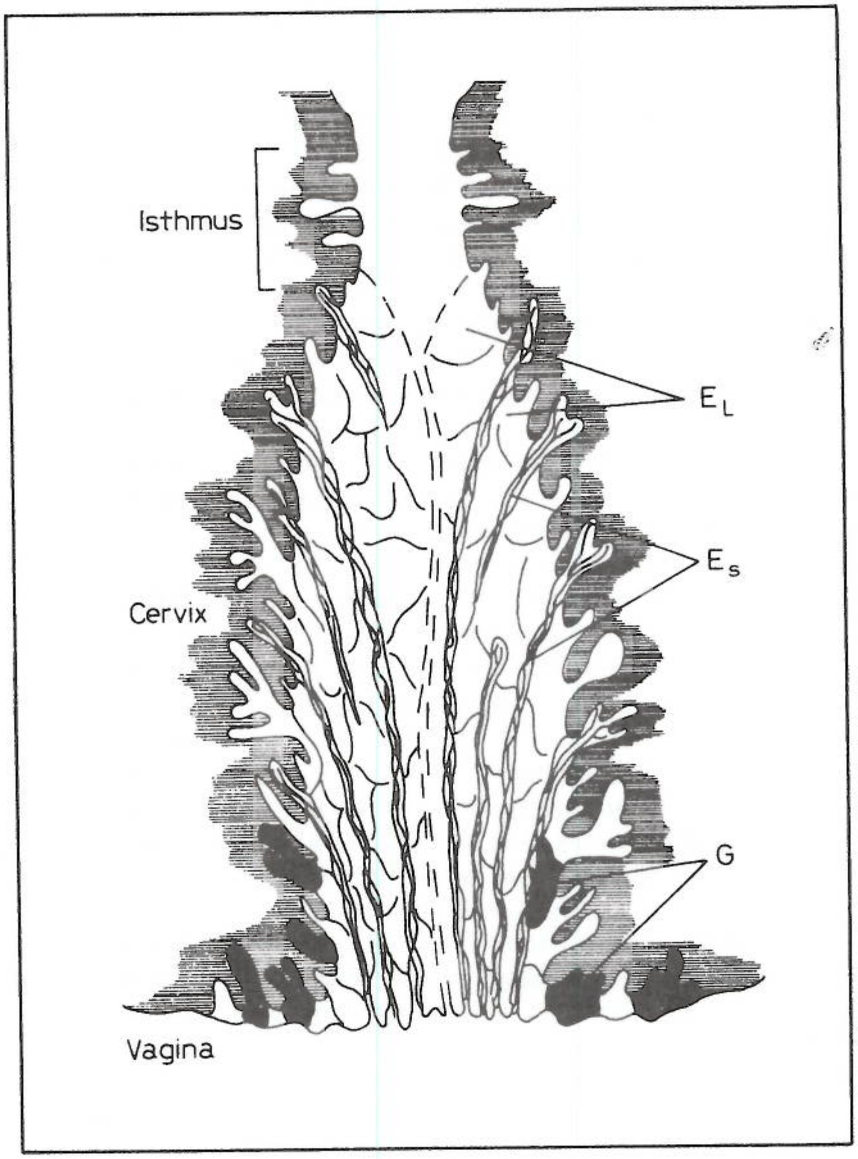

Fig. 1. A schematic view of the architecture of the mucus system of the cervical canal during the ovulatory phase. The irregular lobes of E_L material are illustrated, as well as the long threads of E_S mucus. It should be noted that this system is dynamic and is continuously rebuilt with a time constant of about half a day. This is also illustrated in the picture as E_S strings just being released from some crypts and partially extended downwards in the cervical canal. The location of the G type of mucus mainly in the lower part of the canal is also indicated. It is important to recall that the architecture shown in this figure exists only during 4-6 days around the time of ovulation. The situation during other cyclic phases is not yet known in detail.

Regarding the intermicellar space system, the following considerations apply. The E_S type of mucus contains DT spaces (directed, sperm-transporting spaces) approximately 4 μm wide. The 20-25% E_S mucus is therefore characterized by about 23/4 X 4 = 1.5 as a relative occurrence number. The E_L mucus contains DN spaces (directed but nontransporting spaces, approximately 1 μm wide). Their relative occurrence number is about 75/1 X 1 = 75. The 3% G mucus contains NO spaces (nondirected, nontransporting space) approximately 0.5 μm wide, the relative space-occurrence number being about 3/0.5 X 0.5 = 12.

The total occurrence figure is therefore 1.5 + 75 + 12 = 88.5. The DT channels in the mucus are thus only about 2% (1.5/88.5 = 2) of the total number of spaces. This theoretical consideration is in agreement with empirical counting of the number of spaces (*Odeblad* 1976).

Another important difference between E_S and E_L mucus is the protein content. It is low in the intermicellar spaces of E_S mucus. Thus, the very low viscosity of E_S intermicellar fluid permits very rapid sperm-swimming.

The first important step in the sperm-propagation process is when the sperm cross the borderline between the vaginal contents and the mucus. This crossing occurs to a large extent at the lower end of the E_S-containing strings. Colpomicroscopic observations indicate that the phalanx formations described *in vitro* by *Moghissi et al.* (1964) also may occur *in vivo*. It seems that only a limited number of sperm are permitted to enter a string. This number amounts to 10-150 in each string (0.1-0.3 mm in diameter). *In vitro* studies on mucus explants show that inside each string, the sperm rapidly move "upwards." After some time, there develops a specific mechanism of the propagation process. One or, occasionally, two or three sperm temporarily take on a leading position within the sperm assembly. Observations show that the leading cell(s) retains a leading position(s) only for a short time (order of seconds) and is (are) replaced by some other sperm(s). It is also observed that some sperm slip behind the bulk of the assembly, and that also these slow sperm are replaced by others.

In addition, some sperm also completely lose their motility and come to a permanent stop. A more careful analysis of what really happens within the sperm collection shows that the advancement of the sperm assembly as a whole in each string can be described in terms of mathematical group therapy. Group theory deals with collections of "elements" and their relationships, as given by the so-named group axioms. An extensive literature exists on group theory and its physical and chemical applications. However, common finite groups used in chemistry and physics cannot be directly used in biology. By extending common cyclic groups to (1) so-named concealed groups, and (2) a certain type of paracyclic behavior (infracyclic groups), it is possible to completely describe the sperm propagation within a string. A very short description of groups and their present applications are given in the Appendix.

At ovulation, the number of strings reaching the external uterine os is approximately 400. Each string can primarily accommodate some 100 sperm; the total amount of sperm invading the cervix should be of the order of 40,000. This figure is certainly too low, because invasion also occurs in the less-effectively transporting E_L material; so, totally, about 100,000 sperm may invade the cervix. It may happen that the E_L material may also convey sperm but that this process goes much more slowly. Totally, considerably less than 1% of the ejaculated sperm may enter the mucus. This figure is in approximate agreement with the figure 1:2,000 given (*Settlage et al.* 1973) for the fraction of invading sperm.

TABLE 1
A PRELIMINARY CLASSIFICATION OF SOME IMPORTANT SECRETORY DISORDERS OF THE CERVIX

Type Class	Common Diagnosis
A Disorders affecting single secretory units with reduced cyclic response	
Iso - E units	
Iso - G units	
Iso - V units	acute cervicitis
Iso - Q units	chronic cervicitis
B Disorders affecting more or less the whole secretory system	
E_S hypomucorrhea E_L hypomucorrhea	cervical hypoplasia
E_S hypermucorrhea	hyperestrogenemia
E_L hypermucorrhea	polycystic ovaries cervical hyperplasia
G hypermucorrhea	corpus luteum cyst
V hypermucorrhea	acute cervicitis
Q hypermucorrhea	chronic cervicitis

Pathology

The aspects of the functional pathology of the cervix have to be partially revised in the light of the occurrence of the E_S and E_L components in the ovulatory mucus. Up to the present time very little has been done in this new field. Besides the occurrence of isounits of different kinds (see above), E_S, E_L, and G components can in principle be subjected to amucorrhea, hypomucorrhea, or hypermucorrhea, and V and Q hypermucorrhea may exist. Table 1 contains some information on the various conditions.

In at least one case recently studied there seems to be a complete lack of the E_S type of mucus. This finding means, however, practically nothing until we know if there is some hormonal condition behind the E_S amucorrhea.

If the H_2 type of cervical mucus is very similar to or identical with E_S mucus, the condition of profuse cervical-mucus secretion could be classified as E_S hypermucorrhea.

Using the same terminology, a chronic inflammatory cervical condition should be named Q hypermucorrhea and an acute cervicitis V hypermucorrhea. It may seem that these ways to characterize the cervical fluor is just a matter of terminology and no more. However, the suggested labelings focus the attention on the mucus itself, which is, as stated in the introduction, the medium that all sperm have to pass through. Thus, for fertility and sterility purposes, this new notation has some relevance.

One important fact to point out is that it is not yet clear which hormonal factors stimulate the biosynthesis of the E_S and E_L mucus types. Until this has been clarified, the understanding of these conditions must be inadequate.

Therapy

Modern aspects on therapy of cervical disease have been extensively treated in the recent book on the cervix edited by *Jordan and Singer* (1976). However, the therapeutic aspects related to the E_S-E_L structure of ovulatory mucus have not been possible to include in that book.

The therapy must be intimately related to our knowledge of physiology and pathology. As stated above, the hormonal physiology of the E_S-E_L mucus types is not known so far, and there is no rational basis for therapy of their disorders. If disorders of the E_S-E_L systems are associated with reduced fertility, an adequate treatment of these conditions must await the basic knowledge required.

Insemination Procedures

One important role of the colonization of crypts by sperm seems to be the prolongation of the effect of a single coitus or insemination. The very rapid transport of sperm to the uterine cavity and tubes reported by several authors is not in contradiction with the findings that sperm invade and hibernate in the crypts. The two mechanisms seem to be complementary, assuring a prolonged release and exposure of the ovum to sperm.

With regard to this probable role of the E_S mucus system, insemination should be performed so that sperm are deposited at the external os. The sperm are allowed to colonize the crypts and become slowly released for exposing the ovum. With regard to these aspects, the sperm should not be injected into the cervix or the uterine cavity.

Note Added in Proof

The development in the new E_S-E_L approach to cervical-mucus structure is rapid. Comparison between postcoital and *in vitro* studies on sperm penetration indicates that the effective *in vivo* contact time (= invasion period) between the sperm pool and the cervical mucus in the human is of the order of six minutes. During this short invasion time, essential properties of the sperm-progression patterns are formed, such as the total number of invading sperm, the distribution along the string, and the group behavior of the sperm collection. Regulatory mechanisms of great importance are active, aiding in building up the highly ordered system of penetratory sperm in the strings.

Evidence has also accumulated indicating that irreversible thermodynamics may play a role in assisting energetically the propagation of sperm in the strings.

It can, *a priori*, be expected that these subtile mechanisms may be sensitive to pathological alterations in the cervical mucus, the vaginal contents, the ejaculate, and the borderlines between these compartments.

After submission of this manuscript, a Workshop Conference on the Uterine Cervix in Reproduction was held in Rottach-Egern (sponsored by Organon). A conference report edited by *V. Insler and G. Bettendorf* has appeared in Georg Thieme Publ., Stuttgart. This book contains a contribution from our department, entitled "Sperm Penetration in Cervical Mucus, a Biophysical and Group-Theoretical Approach," by *A. Höglund and E. Odeblad.*

The conference report also contains a paper, "Laser Light-Scattering Studies of Cervical Mucus," by *W. Lee, R. J. Blandau, and P. Verdugo.* In their conclusion the authors write: "Evidence from laser light-scattering spectroscopy does not support the three-dimensional cross-linked molecular network model of cervical mucus reported previously," with reference in the text essentially to the old model presented by myself in which the distinction between E_S and E_L units was not yet established. I should like to point out here that, according to my opinion, there is, in reality, little or no contradiction between the approach of *Lee et al.* and ours. I believe the two aspects on the mucus contain complementary information to our total view on the structure and function of cervical mucus and sperm penetration. A detailed synthesis of the two approaches will be reported on a later occasion.

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Appendix

Basic Knowledge on Group Theory and Its Present Application

A finite group (G, *) consists of a finite number of so-named elements A, B, C, . . . which are interrelated by the four group axioms. The unit element is denoted E, and * is the notation for composition of group elements.

The four group axioms are: (1) (A * B) * C = A * (B * C): this is the associative law; (2) A * B = D: D also belongs to the group; (3) A * E = E * A = A: this equation contains the definition of unit element E; (4) $A * A^{-1} = E$: A^{-1} is the inverse element to A, and A_{-1} also belong to the group.

These laws are valid for all elements of the group. In the present application the elements are either velocity or position of individual spermatozoa. Each of these two parameters forms a finite group in any of these groups; the composition * is arithmetic addition.

One very evident general application of groups is the symmetry of a plane (two-dimensional) polygon, circumscribed by a circle with a constant radius. If this polygon is viewed from the side, the symmetry is not apparent and becomes concealed.

The groups used in the application to sperm transport are equivalent to symmetry groups in a six-dimensional space with the radius of the circumscribed supersphere being constant. The dimensions are x, y, z (metric coordinates), t (time), E (energy), and D (cell density). The symmetry is partially concealed in two dimensions, x and y (perpendicular to the string direction). The order of elements in a cyclic group is successive along the circumscribed circle. If the order is changed, the group property is retained. The cyclic group is, however, changed to the paracyclic group (or, more precisely stated, the type of paracyclic group that is called an infracyclic group).

Now, we are able to apply group theory (within experimental errors) to sperm propagation in a string. As mentioned, we use an infracyclic group of six dimensions (x, y, z, t, E, and D) with two of the dimensions, x and y, partially concealed. All of the dimensions are mutually orthogonal. The dimensions are: x and y perpendicular to string direction, z parallel with string direction, t = time, E = energy, D = cell density. The choice of origo is immaterial. The fact that both position z and velocity dz/dt along the z coordinate follow the group axioms is very important. The only classes of mathematical functions with this property are: (1) cyclic functions, (2) exponential functions, and (3) a combination of these.

Empirical analysis of sperm propagation shows that situation (3) is valid. The cyclic component is the infracyclic group just described and reflects the concealed infracyclic behavior. The exponential component occurs partly as the slowing down of the average penetration rate of the sperm assembly in each string, and partly as a loss of total cell density by sperm immobilization.

All the parameters used to describe the group behavior may have clinical significance; some of the most important are: (1) cycling time, (2) average penetration rate, (3) the slowing down of penetration rate, (4) the fractional death of moving sperm, and (5) the original number of sperm in each string.

For an introductory survey on group theory the reader is referred to the textbook by *Joshi* (1973).