Antibacterial Action of Human Cervical Mucus. (27678)

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Several workers have ascribed antibacterial properties to human cervical mucus. Barton and Wiesner(1) found that cervical mucus sometimes produced zones of growth inhibition of staphylococci in agar plates. Pommerenke(2) reported the inhibitory action of cervical mucus on *Streptococcus hemolyticus* and *Staphylococcus aureus* and stated that hemolytic streptococci and staphylococci incubated for 2 hours with cervical mucus and subsequently plated out on solid media yielded 70-80% fewer colonies than untreated control cultures. No other reports on this subject could be traced in the available English literature.

Our aim in this study was to reinvestigate this reported antibacterial activity and if possible to isolate the substance or substances responsible for it. The bacterial flora of the mucus were also investigated.

Material and methods. The cervical mucus from 110 healthy women between 18 and 40 years of age was examined. Mucus was taken between the 10th and 17th day of the menstrual period, *i.e.*, near the time of ovulation when mucus is more copious, clearer and less viscous.

The cervix was cleaned with gauze, and the mucus aspirated by a sterile tuberculin syringe introduced into the cervical canal. Only women with a healthy cervix, with no erosion or purulent discharge, were selected.

The bacterial flora were examined in 85 specimens by inoculating part of the mucus into Difco Thioglycollate broth. Positive cultures were subinoculated on blood agar and chocolate agar and incubated aerobically and anaerobically.

Tests for antibacterial activity. All samples were preserved at 6°C until examined. The selection of a suitable technique was difficult due to the viscous nature of the mucus. Even the most clear and "fluid" samples were hardly miscible in fluids and could not be diluted for exact quantitative tests. Various attempts to liquefy the mucus were unsuccessful. Treatment with hyaluronidase or with hyaluronidase together with 0.05 NaOH as described by Pommerenke was of no avail. Liquefaction could be obtained by adding 1N NaOH or 1N H_2SO_4, but since these reagents might themselves destroy the antibacterial substance this treatment was not considered desirable. Another drawback of this method is the necessity of neutralizing the mucus after liquefaction, so that the substance looked for is unduly diluted. The following technic was finally adopted: Circular holes, 5 mm in diameter, were punched in agar plates inoculated with the organisms examined. The holes were filled with mucus and the plates incubated at 37°C. In the latter part of the study 2-3 drops of a 1 mg/ml solution of lysozyme chloride (crystalline, egg white, product of L. Light & Co.) were introduced into an additional similar hole.

After 24 hours incubation the plates were inspected for the appearance of zones of inhibition.

The following micro-organisms were tested: *Staphylococcus aureus* (coagulase positive), *Staphylococcus albus* (coagulase negative), *Streptococcus hemolyticus, Streptococcus fecalis, Escherichia coli, Micrococcus lysodeicticus, Sarcina lutea,* and *Bacillus* sp.

Difco nutrient agar pH 6.8 was used throughout experiments unless otherwise specified. A number of comparative tests were performed on agar plates adjusted to pH 5.2, 6.0, 7.0, 8.2, and 8.5. Blood agar was utilized for tests with hemolytic streptococci.

Results. The pH of the mucus measured with a paper indicator was 9-9.5.

Flora. About a third of the samples examined were found to be sterile. The other two-thirds contained rather scanty flora, the most frequent organism being the lactobacillus. This was apparently a contaminant from the vagina. Other bacteria found were diphtheroids, coagulase-negative staphylococci,

non-hemolytic streptococci, *E. coli,* and yeasts.

Antibacterial action. Twenty samples of mucus tested for a growth-inhibitory effect on *Staph. aureus* and *Staph. albus,* 8 samples tested with *Strep. hemolyticus* and *Strep. fecalis,* and 4 samples tested with *E. coli* all gave negative results with both cervical mucus and lysozyme, no zone of inhibition being observed. Growth of *M. lysodeicticus,* however, was inhibited by all of the 40 mucus samples tested against this organism, as well as by lysozyme. It thus seemed probable that lysozyme or a lysozyme-like substance was present in cervical mucus.

Zones of inhibition produced by mucus were from 10 to 22 mm in diameter; those by lysozyme from 17 to 25 mm. *S. lutea* was inhibited by 2 samples of mucus in a zone of 20 mm and by lysozyme in a zone of 21 mm diameter. Aerobic sporogenic bacilli behaved similarly. Two aerobic sporogenic bacilli which had contaminated the test plate were examined with 8 samples of mucus. A zone of inhibition varying from 10 to 16 mm was produced. Lysozyme similarly tested caused an inhibition zone of 17 to 22 mm.

The clear inhibition zones produced by both lysozyme and mucus continued to spread after additional incubation. This further clearing is characteristic of the process of dissolution of bacteria under the impact of a diffusing enzyme.

The influence of temperature on antibacterial activity of the cervical mucus in comparison with that of lysozyme was examined as follows:

Pooled mucus was divided and transferred to test tubes. 0.8 ml of a 1 mg/ml solution of lysozyme was introduced into each of a second series of tubes. The tubes were incubated for 15 or 30 minutes in a water bath at various temperatures and the contents tested, as before, against *M. lysodeicticus.* The results are seen in Table I.

The antibacterial agent in cervical mucus is thus heat-labile, being partially destroyed at 70°C and totally at 80°C. The lysozyme preparation used was comparatively heat-stable.

Dialysis. A cellophane bag containing approximately 1 ml of pooled mucus was suspended in 2 liters of distilled water cooled with ice. The dialysis lasted 2 days, the distilled water being changed after 24 hours. On the third day the mucus was tested for inhibitory activity against staphylococci and *M. lysodeicticus.* A zone of inhibition of 12 mm was produced round *M. lysodeicticus* only. This result supports the suggestion that the antibacterial substance is a lysozyme-like protein.

TABLE I. Influence of Temperature on Antibacterial Effect of Cervical Mucus and Lysozyme.

		Zone of inhibition of *M. lysodeicticus* in mm	
		Lysozyme	Mucus
Before heating		23	18
After 30 min at	60°C	22	15
	70°C	*	*
	80°C	21	0
	100°C	19	*
Before heating		25	23
After 15 min at	60°C	25	21
	70°C	25	13
	80°C	25	0
	100°C	*	*

* Not examined.

Comparison of lytic actions. The following test was performed to compare the lytic action of cervical mucus with that of lysozyme. A 48-hour-old culture of *M. lysodeicticus* grown on Difco Stock agar was washed with a phosphate buffer of pH 7.0. Lysozyme and mucus respectively were added to the bacterial suspensions. Changes in optimal density were measured in a Klett photocolorimeter at 540 mμ wave length. Similar experiments were done using a culture of *E. coli.* The results are shown in Table II.

TABLE II. Effect of Cervical Mucus and Lysozyme on Optical Density of Suspensions of *M. lysodeicticus* and of *E. coli.*

	Optical density			
	Time			
Bacterial suspension	0	10 min	30 min	90 min
M. lysodeicticus +				
Cervical mucus	182	126	99	76
Lysozyme, 40 μg/ml	185	69	69	65
Control	187	186	186	182
E. coli +				
Cervical mucus	200	200	200	196
Lysozyme, 40 μg/ml	182	182	182	180
Control	183	182	182	181

Protective effect of sucrose. One of the properties of lysozyme is to convert sensitive bacteria to protoplasts. Therefore if lysozyme is the active substance in cervical mucus, bacteria acted upon by mucus should also be converted to protoplasts. Sucrose on the other hand should protect the bacteria against lytic action. The following experiment showed this to be the case.

A 48-hour-old culture of *M. lysodeicticus* grown on brain heart agar at 37°C was harvested, washed 3 times in distilled water, and suspended in 1 M sucrose + 0.05 M sodium chloride(3). To 2.5 ml of this suspension 0.4 ml of mucus was added and mixed with a pipette. The mucus mixed quite well but the suspension became somewhat viscous. 0.2 ml of this suspension was added immediately (time zero) to 4 ml of distilled water and to 4 ml of a sucrose solution respectively.

Optical density was measured in a Unicam Spectrophotometer at 500 mμ wave length. Samples were examined after 3 and 20 hours (Table III).

TABLE III. Protective Effect of Sucrose on Lytic Action of Cervical Mucus.

	Optical density		
	Time 0	3 hr	20 hr
Suspension of *M. lysodeicticus* and mucus			
in distilled water	220	75	50
in sucrose solution	120*	115	112

* The initial low optical density of the suspension prepared in sucrose is due to the high refractive index of this solution (4).

Comment. Thompson(5) in reviewing the relation of lysozyme to the antibacterial properties of various tissues and secretions, does not mention cervical mucus as a source of lysozyme. His only reference to lysozyme in the female genital tract is the statement based on his unpublished data, that "vaginal washings contain small quantities."

The number of leucocytes in cervical mucus taken at time of ovulation is scanty(6), so that lysozyme seems to be a constituent of the mucus itself and not a product of leucocytes contained in the mucus. In human seminal fluid in addition to lysozyme(7), other antibacterial substances, namely spermine and spermidine are present(8,9). In cervical mucus no antibacterial substance other than lysozyme was found.

Although no chemical antibacterial substances active against pathogenic bacteria have been found in human cervical mucus, the latter may nevertheless play an important role in defense of the uterus against infection. Due to its physicochemical composition cervical mucus presents an inhospitable terrain and a mechanical barrier, and is thus impervious to attack by occasional contaminant bacteria.

Of a series of bacteria tested only *Serratia marcescens* was able to decompose mucus (10).

Summary. The antibacterial action of human cervical mucus was investigated. The results indicated that cervical mucus contains a lysozyme-like substance.

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