Immunochemical Study of Vaginal Secretion. I. Detection of Serum and Non-Serum Protein Components in Normal Vaginal Secretion.* (28274)

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Detection of carcinoma of the uterus and of the cervix by cytologic and cytochemical examination of vaginal mucus has been extensively studied, but little attempt has been made to detect changes that occur in chemical and immunologic reactivities of the humoral phase of vaginal secretion in neoplastic disease of the uterus. Although increases in the activities of β-glucuronidase(1,2) and of α-mannosidase(3) have been reported, they are not recommended as a test for diagnosis of cancer of the cervix because of their lack of specificity. Recent studies in this laboratory have demonstrated the prevalence of serum proteins in gastric juice(4) and in bronchial secretions(5,6). Of special interest during these observations, however, was the finding of certain antigenic components not present in normal human serum. Preliminary studies on the immunochemical analysis of vaginal secretions from normal women are hereby reported.

Materials and methods. Specimens of vaginal secretion were collected at the Earl and Bessie Whedon Cancer Detection Foundation, Sheridan, Wyoming, from 55 healthy adult volunteers. A Clay-Adams vaginal pipette with rubber bulb was used to collect the material. Specimens were obtained during the mid-menstrual cycle 10 days after termination of the menstrual flow and at least 5 days prior to the expected period. Specimens were frozen at −10°C and stored at this temperature until tested.

Double diffusion-precipitin analysis of the vaginal secretions was made with a modified Ouchterlony technique(4). Immunoelectrophoresis was carried out with Scheidegger's micromodification(7) using veronal buffer, pH 8.2, $\mu = 0.05$, and buffered 1.5% Difco Bactoagar. A potential gradient of 90-110 volts and 3.5-4.0 ma per slide was applied for 3.5 hours.

The following antisera were employed in each of the above precipitin methods:

Antisera	Antigen	Code No.
1) Horse	Normal human whole serum	H-IEP #65 (Hyland)
2) Rabbit	Crystalline human serum albumin	R-100 + 104 (Hyland)
3) "	Human Cohn fraction II	R-38 B (Hyland)
4) "	Pooled sputum from patients with bronchogenic carcinoma	ABCS
5) "	Pooled sputum from patients with non-neoplastic respiratory diseases	ANCS

The methods used for production of the anti-sputum sera have been described(5). Either the unabsorbed antiserum, ABCS, or that absorbed with normal human serum proteins was used. The latter absorbed antiserum was designated as ABCS-a.

Results. 1. *Double diffusion-precipitin analysis.* When the vaginal specimens were allowed to react with the anti-serum H-IEP #65, 13 out of 55 (23.6%) gave a precipitin reaction (Tables I and II). Several of the remaining 42 specimens, however, were not entirely free from serum proteins as shown by precipitin reactions with antisera R-100 + 104 and R-38 B in 23 (41.8%) and in 28 (50.9%), respectively. When antisera to bronchial secretions, *i.e.*, ABCS and ANCS, were allowed to react with the same specimens, 38 of 55 specimens (69.1%) in each case gave positive precipitin reactions. When the antiserum, ABCS-a, was tested, positive reactions were observed in 18 of 55 specimens (32.7%).

No precipitin line could be found with any of the 6 antisera used in 15 cases (27.3%).

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TABLE I. Double Diffusion-Precipitin Analysis of Vaginal Secretions from Healthy Volunteers.

No. of cases	H-IEP #65	R-100 + 104	R-38 B	ABCS	ANCS	ABCS-a
15	—	—	—	—	—	—
1	—	—	—	+	—	—
1	—	—	—	—	+	—
1	—	—	+	+	—	—
1	—	—	+	—	+	—
7	—	—	—	+	+	—
2	—	—	—	+	+	+
3	—	—	+	+	+	—
1	—	—	+	+	+	+
6	—	+	+	+	+	—
2	+	+	+	+	+	—
1	+	+	—	+	+	+
4	—	+	+	+	+	+
10	+	+	+	+	+	+

— = Negative precipitin reaction.
+ = Postive precipitin reaction, including from faint trace to strongly positive reactions.

TABLE II. Double Diffusion-Precipitin Analysis of Vaginal Secretions from Healthy Volunteers.

Reactions	H-IEP #65	R-100 + 104	R-38 B	ABCS	ANCS	ABCS-a
—	42	32	27	17	17	37
±	1	5	2	4	3	5
+	7	6	15	19	18	10
++	3	9	6	8	9	2
+++	2	3	5	7	8	1

± = Faint trace reaction.
+, ++, +++ = Positive precipitin reactions, classified according to an arbitrary standard which is based upon number and density of the precipitin arcs.
Figures indicate No. of cases.

On the other hand, 10 specimens (18.2%) reacted with all of the 6 antisera. In 2 instances, all antisera except ABCS-a gave positive precipitin reaction. One specimen formed precipitin lines with all antisera except with R-38 B. Eleven specimens (20.0%) did not react with the 3 anti-serum protein sera, H-IEP #65, R-100 + 104 and R-38 B, yet reacted with the anti-sputum sera. No precipitin lines were formed when the remaining 16 vaginal specimens were allowed to react with antiserum H-IEP #65. In case of the 4 antisera R-100 + 104, R-38B, anti-serum to specific serum fractions and anti-sputum sera, positive precipitin reactions were observed in every instance. Moreover, none of the specimens which failed to react with the anti-sputum sera reacted with the anti-serum protein sera.

2. *Immunoelectrophoresis.* Sixteen specimens exhibiting strong precipitin reactions in the double diffusion agar plates were subjected to immunoelectrophoresis using the antisera H-IEP #65 and ABCS. When allowed to react with H-IEP #65, 11 of the 16 (68.7%) yielded a distinct precipitin arc due to albumin. Of these, 7 (43.7%) also showed a precipitin arc due to γ-globulin. Precipitin arcs due to α_2-, β_1- and β_2-globulins were also found in some of the specimens (Table III, Fig. 1-A).

Immunoelectrophoretic patterns obtained by reaction with ABCS had in general more precipitin arcs than those allowed to react with H-IEP #65 (Table III, Fig. 1-B, 2-A).

Identification of the precipitin arcs other than albumin, β_1-siderophilin, γ-globulin, β_{2A}-globulin and β_{2M}-globulin, was difficult because the precipitin patterns were different in many respects from those obtained by reaction of normal serum and the same antiserum. In the latter case very conspicuous

TABLE III. Immunoelectrophoretic Analysis of Vaginal Secretions.

	H-IEP #65							ABCS						
No.	Pre-alb	Alb	α_1	α_2	β_1	β_2	γ	Pre-alb	Alb	α_1	α_2	β_1	β_2	γ
1		1		1	1		1		1		2	1	2	1
2		1					1		1		3	1	3	1
3		1							1		1	1		
4		1				1	1		1		1		2	1
5		1					1		1		1	2	1	1
6		1					1		1		2	1	2	2
7		1		1	1		1		1	2	3		3	1
8													1	
9														
10		1							1				2	
11									1		2		1	1
12		1							1		2		1	1
13													1	
14		1		1			2	1	1	3	2	1	3	1
15														
16		1							1	1		1		1
Total*	0	11/11	0	3/3	2/2	1/1	8/7	1/1	12/12	6/3	19/10	8/7	22/12	11/10

Figures indicate No. of precipitin arcs observed in the regions.
* No. of precipitin arcs observed/No. of cases having positive reaction.

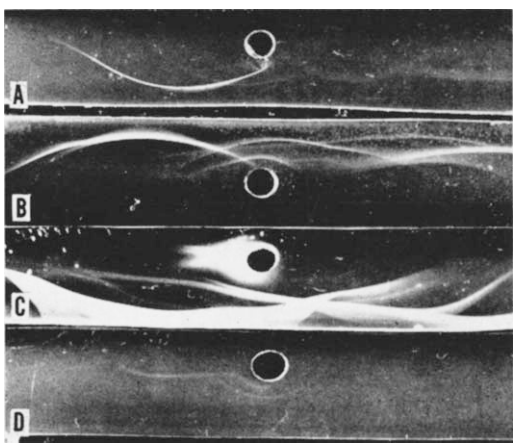

FIG. 1. Immunoelectrophoretic patterns of vaginal secretions and of serum. Anode on left side. A. Vaginal secretion reacted with H-IEP #65. B. Vaginal secretion reacted with ABCS. C. Normal human serum reacted with ABCS. D. Vaginal secretion reacted with ABCS-a.

precipitin arcs due to albumin, γ-globulin, and β_{2A}-globulin, together with several minor precipitin arcs in α- and β-globulin regions, were observed (Fig. 1-C).

When vaginal secretions were allowed to react with the absorbed antibronchial secretion serum, several precipitin arcs were still present (Fig. 1-D and 2-D).

The immunoelectrophoretic patterns of pooled bronchial secretions from patients with bronchogenic carcinoma and of gastric juice from patients with carcinoma of the stomach (anacid, pH 7.2) were compared with the patterns of vaginal secretion. When allowed to react with the anti-serum ABCS, each of the 3 secretions gave a unique pattern, although each showed at least one antigenic component which reacted with ABCS-a (Fig. 2). Differences in electrophoretic characteristics of these non-serum protein components were observed, yet an immunologic "reaction of identity" was clearly demonstrated when the 3 secretions were allowed to react with ABCS-a in an Ouchterlony agar plate (Fig. 3).

Discussion. Evidence from the literature establishes the presence of serum proteins in gastric(4) and in other gastrointestinal secretions. Hollander and Horowitz(8) pointed out that such observations indicated that the efflux of the serum proteins into the alimentary tract might be a fairly general physiologic phenomenon. Recent studies by the authors demonstrated a high incidence of serum protein components in bronchial secretions. Therefore the finding of serum proteins in another mucous secretion, *i.e.*, vaginal secretion, was not unexpected. Results of double diffusion-precipitin analysis, using the antiserum H-IEP #65, demonstrated the presence of serum proteins in approximately one-fourth of the vaginal secretion specimens. Use of more sensitive antisera to particular fractions of serum proteins demonstrated that approximately one-half of the specimens contained

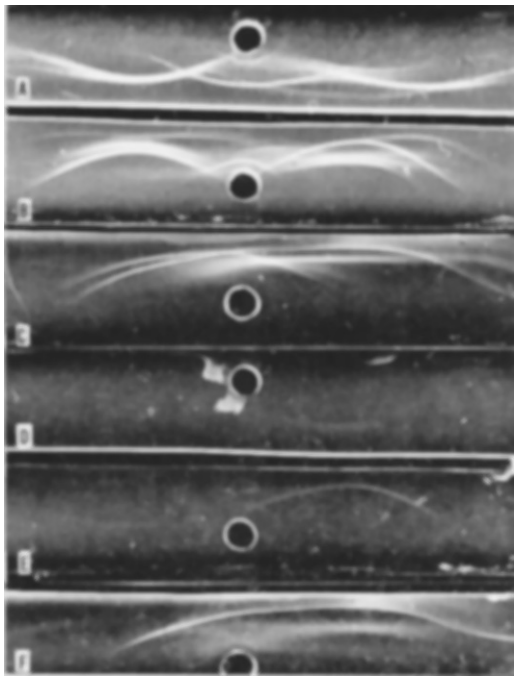

FIG. 2. Immunoelectrophoretic patterns of vaginal, bronchial, and gastric secretions. Anode on left side. A. Vaginal secretion reacted with ABCS. B. Bronchial secretion reacted with ABCS. C. Gastric juice reacted with ABCS. D. Vaginal secretion reacted with ABCS-a. E. Bronchial secretion reacted with ABCS-a. F. Gastric juice reacted with ABCS-a.

serum albumin and/or serum γ-globulin. Discrepancies between the reactivities of the specimens with the 3 different antisera to human serum proteins and their fractions may reflect the difference in antibody titers of the antisera. The over-all incidence of serum proteins in vaginal secretions, therefore, may be even higher than was detected in the present study.

The higher incidence of positive precipitin reaction with the anti-sputum sera than with anti-serum protein sera likewise may be explained by the difference in antibody titers between these 2 antisera. Previous observations with sputum antigens, however, revealed that the anti-sputum sera contained antibodies to proteins not present in human serum, in addition to antibodies to normal human serum proteins. Approximately one-third of the vaginal specimens reacted with the anti-serum ABCS-a, indicating presence of antigenic proteins having cross-reactivities with the "sputum-specific proteins." The cross-reacting antigenic protein was also detected in anacid gastric juices from patients with carcinoma of the stomach. Immunoelectrophoretic analysis demonstrated that the components of the 3 different secretions that reacted with the antiserum ABCS-a had different electrophoretic mobilities in spite of their immunologic cross-reactivities. There is the possibility in the case of gastric juice that the antigenic component had undergone a degradation due to enzymic digestion, although the anacid gastric juice had only negligible peptic activity. Effect of peptic digestion, however, cannot be implied in the cases of bronchial and vaginal secretions. Therefore, it is concluded that the 3 epithelial secretions under study contain antigenic components with different physicochemical properties. Yet they possess an antigenic determinant, or determinants, common to all.

In the present study, anti-sputum sera were employed for detection of components other than serum proteins. Use of antisera produced by immunization of animals with normal and pathologic vaginal secretions may disclose still other components specific for the vaginal mucus. In the study, only specimens from healthy subjects were used. Analysis of

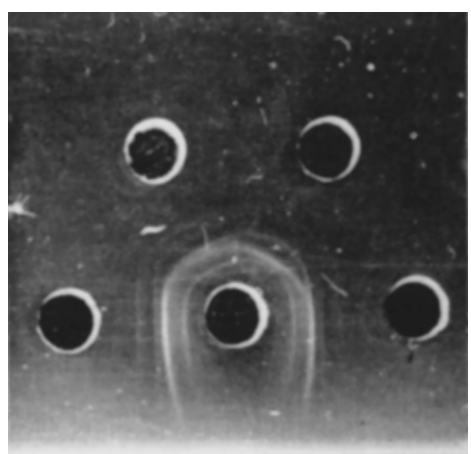

FIG. 3. Double-gel diffusion plate showing reactions of identity between vaginal, bronchial, and gastric secretions. Center well, lower row, contained the antiserum, ABCS-a. Antigen wells contained: Upper row, left, pooled bronchial secretion; right, pooled gastric secretion. Lower row, pooled vaginal secretions at left and right of antiserum well.

vaginal secretions from patients with carcinoma of the cervix are in progress in an effort to determine if specific components can be detected which may be utilized in the immunological diagnosis of the disease.

Summary. Vaginal secretion from 55 healthy adult volunteers were analyzed with the double diffusion-precipitin test using antisera to serum proteins and antisera to bronchial secretions, as a preliminary study in search of an immunological means of detecting cancer of the cervix. Approximately one-half of the specimens contained detectable amounts of serum proteins, while 70% reacted with the anti-sputum sera. Of these, 18 specimens (32.7%) reacted with the anti-sputum serum absorbed with normal human serum proteins. Characterization of the components reacting with the antisera was achieved with the immunoelectrophoretic study, and the immunoelectrophoretic patterns of vaginal secretions were compared with the patterns of gastric juices and of bronchial secretions. The 3 secretions had components having cross-reactivities although their physico-chemical properties were different. Use of antisera to vaginal secretions and application thereof to pathologic vaginal secretions are indicated.

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Determination of Tris-(Hydroxymethyl) Amino Methane (THAM).* (28275)

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Since the original description of the effects of administration of the organic buffer Tris-(hydroxymethyl) amino methane (THAM) *in vivo*(1) considerable interest has arisen in its potential usefulness as a pharmacological agent(2). The fact that administration of large doses of Tris-(THAM) may cause serious reactions makes desirable the study of its absorption and excretion. Determination of plasma and urine levels of this compound has been limited, however, by lack of a simple, reliable method.

To the authors' knowledge, 5 methods have been described for the determination of Tris-(THAM). 1. Accounting of C-14 labeled Tris-(THAM)(3). 2. Oxidation by potassium dichromate(4). 3. Oxidation to ammonia by alkaline periodate(5). 4. Conversion to ammonia and determination with a modified sodium phenate method(6). 5. Determination by the naphthoquinone method for amino acids(6).

Some of these methods are of limited value for general use, since they require elaborate equipment. Of the simpler methods, details of the procedures are lacking, as are data on the validity of their application to plasma or urine.

This paper outlines a method based on that of Rosen(4) and presents results of the application of this modified method and its evaluation with respect to potentially interfering substances.

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