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Occurrence of Antibody in Human Vaginal Mucus.* (26421)

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The occurrence of specific antibody activity outside the tissues proper and often contained in mucous secretions has been described in a number of mammalian species. Pierce has reviewed critically much of the relevant material(1). He refers to such antibody as mucoantibody, implying only that the activity is found to occur admixed with mucoprotein. Such antibody found in saline extracts of feces, and presumably occurring free in the bowel, has been called coproantibody.

The evidence suggests that such antibody in the upper respiratory tract is closely related to, and possibly derived from, circulating antibody. In other instances, notably in the case of antibody found in feces, vaginal secretions, and urine, antibody response following active immunization by the parenteral route appears to differ significantly from serum antibody response in that the activity appears and reaches a peak earlier than serum antibody, and disappears while serum antibody persists. In contrast to serum antibody, such antibody is probably not cumulative, and the observed titers may reflect significantly the rate of antibody formation. Quantitative studies have supported this view(1,2,3), and it is inferred that the antibody is formed locally.

The occurrence of muco-antibody in areas subject to an essentially local infection without invasion of the deeper tissues provides a rational basis for an effective antimicrobial prophylactic immunity, and may also be a factor in the normal microbial flora of such areas. In the first instance, immunity to experimental enteric cholera in the guinea pig (4) and rabbit(5), and similar Shigella infections in the guinea pig(6) and mouse(7) appears to be a function of the presence of antibody in the bowel. Similarly, immunity to Trichomonas infection of the bovine vagina is also associated with presence of antibody in the vaginal mucus(1). In the second, the occurrence of coproagglutinins to coliforms and enteric pathogens in man(8,9, 10) as well as in experimental animals is suggestive in connection with the observed succession of immunologic types of coliform bacilli in man(11).

In addition to the immune response demonstrable in bovine vaginal mucus to Trichomonas infection, antibody to homologous sperm has been found in the rabbit following

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local application of sperm or testis(12), and in the serum and uterine secretions of both rat and rabbit after introduction of homologous and heterologous sperm into the vagina (13). In man the occurrence of anti-sperm agglutinins in the cervical secretions of human females shortly after ovulation and in the second trimester of pregnancy has been reported(14), and hemagglutinins to Group A and Group B red blood cells has been found in the cervical secretions of Group O women(15).

The present report is concerned with the immune response of the human female to conventional typhoid bacillus vaccine given parenterally, and to soluble typhoid bacillus antigen applied locally, demonstrable as agglutinin in serum and vaginal mucus.

Methods. The immunizing agents were commercial typhoid-paratyphoid (TAB) vaccine, and soluble typhoid bacillus antigen. The latter was prepared by sonic lysis (9 KC/sec for 2 hours) of a heat-inactivated (58-60°C for 30 minutes) saline suspension of an 18 hour culture of *Salmonella typhosa* 901 on veal infusion agar. The lysate was cleared by centrifugation at 4500 × G and the supernatant diluted in saline to contain approximately the same amount of protein nitrogen (Folin-Ciocalteau method) as commercial typhoid vaccine, *i.e.*, 50-60 μg/ml. The agglutinating antigen was a saline suspension of living strain 901; *i.e.*, antibody to both H and O antigens was titrated.

Human female volunteers were immunized by subcutaneous inoculation of a single dose of 0.5 ml of typhoid vaccine in the case of the parenterally immunized group, and the second inoculation was given when vaginal mucus agglutinin was no longer detectable even though serum agglutinin persisted. In the immunization of the group receiving antigen locally, a junior-sized Tampax was soaked with 10 ml of the soluble antigen solution, inserted in the vagina, and allowed to remain for 3 days.

Vaginal mucus was collected by swabbing the cervix uteri and posterior vaginal fornix with a small cotton pledglet, slightly moistened with saline. The pledglet was then washed out in 5 ml of saline, the solution clarified by centrifugation at 3000 × G, and titrated for agglutinin. It was assumed that this supernatant represented a 1:5 dilution of vaginal mucus. Mucus and blood specimens were taken prior to immunization and at weekly or biweekly intervals thereafter, but mucus specimens were not taken during the menstrual period. Postimmunization time was measured as days after first contact with the antigen.

Results. The observed mucus and serum antibody response following primary parenteral inoculation with typhoid vaccine is shown in Table I. It is evident from these data that the titer demonstrable in vaginal mucus was closely similar to that of copro-antibody in its relation to serum agglutinin. In spite of unavoidable irregularity in time of taking specimens, agglutinin was demonstrable in the vaginal mucus of 2 of the 5 subjects by the 3rd day and before serum agglutinin could be detected. The muco-antibody titer reached a peak of 1:100 in all subjects 9-20 days after inoculation, remained at approximately this level until the 4th week, and declined thereafter. It had completely disappeared in 3 of the 5 subjects after 7 weeks, persisted for another week in one subject, and to nearly 4 months in another. This last subject, however, proved to have an hypertrophic endometrium with constant slight oozing of blood, and the muco-antibody status of the observed agglutinin is questionable. Serum agglutinin persisted in all subjects over the entire period of observation.

In the secondary response, elicited by a second parenteral inoculation of vaccine after complete disappearance of agglutinin from vaginal mucus, the vaginal mucus agglutinin again appeared as early as the 4th day in 2 of the subjects. While peak titer was first reached in mucus and serum at 20 and 22 days postimmunization on the average in the primary response, in the secondary response peak titers were reached at 23 and 41 days respectively to give a marked discrepancy between vaginal mucus and serum response. Although vaginal mucus agglutinin titers were somewhat higher than in

TABLE I. Agglutinin Response to Parenteral Typhoid Vaccine.

Immune response		Days after inoc.	Subject A (1940)* Mucus	A (1940)* Serum	B (1957)* Mucus	B (1957)* Serum	C (none)* Mucus	C (none)* Serum	D (none)* Mucus	D (none)* Serum	E (none)* Mucus	E (none)* Serum
		0	0	0	0	0	0	0	0	0	0	0
		3	10		10		0		0		10	
		7			10	0	10	10			10	0
		10			50		100				50	
	Primary	17	50	200	100	200	50	1000	100	2000		
		21	100						100			
		24	100	5000			100	500	100	2000	100	500
		28			100		50		100		100	
		31	10	500	10	200	20	500				
		33									100	500
		35	10		10		50				50	
		51	0		0		0		50	1000	50	
		0	0	200	0	500	0	500	0	20	0	
		3	0		0		20		200			
		7	0	200			20	1000	200	1000	100	500
		10	20				20		100			
		14	50	200	20	100	100	500			100	
		17	50		100						200	
		21			100	200					200	
		24			100		100		10	1000	200	
		28					50					
		31	50		50		50		10		200	1000
	Secondary	35	100	500			200		50	500		
		38	10		50		20		20			
		42			100	2000	50	1000			200	
		45	0									
		47									100	
		49	50		10	200			0			
		51									100	5000
		52	20	1000	0		10					
		54									20	
		56			0	100						
		63	0	1000			100	500				
		68									0	
		70					0					
		72									0	5000

* Date of prior typhoid immunization.

the primary response, to as much as 1:200 in 3 cases, they did not persist longer, and, as in the primary response, disappeared while serum titers persisted at levels of 1:100 to 1:5000 (Table I). Of the subjects, 2 had had earlier conventional typhoid immunization, 2 had not, so far as could be determined, and in one case it was not known whether or not there had been prior immunization.

The immune response of a group of 8 subjects to locally applied soluble typhoid antigen appeared to differ significantly from that to parenteral vaccine. Relatively high agglutinin titers, 1:100 to 1:200, were found in 3 subjects in the first mucus specimens taken and at the end of the 3-day period of exposure to the antigen. Peak titers were reached in an average of 15 days and were very high with 7 of the 8 subjects showing an agglutinin titer of 1:2000. In general, serum agglutinin response was inferior to that obtained with parenteral vaccine, except in 2 subjects who had a history of recent vaccination (Table II).

Discussion. In the 2 earlier reports of the presence of antibody in human vaginal mucus noted above, *viz.*, sperm agglutinins and blood group antibodies, the status of the antigenic stimulus is somewhat uncertain in the former and presumably nonexistent in the latter. The study reported here is concerned with a model system in which the antigen is a conventional immunizing preparation, and the microorganism does not nor-

TABLE II. Agglutinin Response to Locally Applied Soluble Antigen.
(Immune response)

Days after inoc.	Subject															
	Gl (1952)*		Hi (1955)*		St (none)*		We (1954)*		Co (none)*		Com (1953)*		Mo (1960)*		Bl (1960)*	
	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S
0	0	0	0	0	0	0	0	0	0	0	0	0	0	500	0	5000
3									100		200				200	
4	0		0		200		2000	5000								
6									200	200	500	2000				
7					100	200	1000									
8	100		200	200												
11	100	500			1000		1000						500	2000		
13			100						500	100	100	2000			1000	
14					100	200	200	500								
15	2000												2000			
17									2000		2000					
18	500	100			2000		500						200	1000		
20									20	20	0	500				
21					0	0										
22	1000		2000	200									1000			
24							20	1000	20		0					
25	20		0										20	1000		
27					100	100			200	1000	50	500			0	500
28	20		0	1000			20						20			
32	200	200	50												50	
43	1000														20	

* Date of prior typhoid immunization.

M = mucus; S = serum.

mally occur in the vagina. The observed results would seem, therefore, to establish the occurrence of specific antibody in human vaginal mucus as a part of the active immune response.

While it is probably not possible to differentiate a primary and secondary response because of the frequency of typhoid immunization and intercurrent infections with Salmonella types having antigens in common with the typhoid bacillus, the evidence presented here suggests that primary and secondary responses do not differ greatly with respect to vaginal mucus agglutinin although they do in serum antibody response. It was of interest too that neither the numerous menstrual periods, nor the 2 pregnancies which intervened in the soluble antigen group, nor the single pregnancy in the secondary response group, had any apparent effect on the rise and fall of antibody titer in the vaginal mucus.

The similarities in behavior of the titer of vaginal antibody in relation to serum antibody to that of coproantibody are striking. Its relatively rapid appearance and disappearance and seeming independence of serum antibody would suggest that here also the nonaccumulative titer may be a measure of rate of antibody formation, and that the observed antibody is formed locally. The case for local formation of vaginal antibody is strongly supported by the superiority of this immune response to locally applied antigen, and the relatively poor serum antibody response to this kind of antigenic stimulus.

Whether or not antibody present in vaginal mucus is a factor in the composition of the normal microbial flora of the vagina, is a significant element in effective immunity to vaginal infections, or even may relate to the viability of sperm, is not clear.

Summary and conclusions. Using typhoid vaccine, soluble typhoid bacillus antigen, and specific agglutinin as a model antigen-antibody system, it has been possible to demonstrate the occurrence of specific antibody in human vaginal mucus as a consequence of active immunization. Vaginal antibody was produced in response to either parenteral or locally applied antigen and the response to the latter kind of antigenic stimulus was superior to that of the former. Vaginal agglutinin appeared earlier than serum antibody, reached a peak slightly sooner, and disappeared in 7-8 weeks though serum antibody persisted, often in relatively high titer. Vaginal antibody response to primary and secondary inoculation appeared to be substantially the same, and was not affected by menstruation or intervening pregnancy.

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