Pediatr. Infect. Dis., 5(1):S148-S151. 1986.

Levels of human milk-specific immunoglobulin A antibodies during lactation

JOSE RAMIRO CRUZ, MSC, DSC AND CAROLINA AREVALO, BS

Human milk contains a variety of antiinfective substances such as lactoferrin, lysozyme, bifidus factor, anti-staphylococcal factor, components of the complement system, and immunoglobulins and cells engaged in immune responses.[1,2] The most important of the soluble substances, in terms of protection against diarrheal disease in the breast-fed child, seems to be the secretory immunoglobulin of the IgA class (SIgA).[3,4] It is accepted that milk SIgA is produced locally by IgA-committed lymphocytes primed in the intestine which, after priming, migrate to the mammary gland in the pregnant or lactating female.[5] Thus, the passive protection of the breast-fed individual against the intestinal pathogens present in the maternal environment is assured. This rationale is supported by the observation that populations living in areas where cholera is endemic have milk antibodies against cholera toxin, whereas women from cholera-free settings do not.[6] Furthermore antibodies against food components of the maternal diet also have been detected in breast milk.[7,8]

Long-term follow-up studies of milk antibodies in human milk have shown that the concentration of IgA specific antibodies fluctuate during lactation.[9,10] These changes seem more dramatic in women living in geographic areas where hygienic conditions are poor. We followed 20 lactating mothers from a rural community in Guatemala (Santa Maria Cauqué) taking daily samples from the 5th to the 9th day postpartum and weekly thereafter for 12–16 weeks. Total SIgA concentration and IgA-specific antibodies against *Escherichia coli*-labile toxin (EcLT), *Shigella* B6 somatic antigen (SB6) and rotaviruses (RTV) were determined by means of the enzyme-linked immunosorbent assay (ELISA). The mean concentration of SIgA followed the expected pattern, being highest at the beginning of lactation, declining rapidly during the first week, to reach a plateau 2 to 3 weeks postpartum. The levels of specific antibodies against EcLT, SB6 and RTV

From the Section of Infection, Nutrition and Immunology, Division of Nutrition and Health, Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Guatemala.

Address for reprints: J. R. Cruz, INCAP, P.O. Box 1188, Guatemala, Guatemala.

INCAP Publication I-1130.

TABLE 1

Number of milk samples with specific antibodies against EcLT, SB6 and RTV

Subject No.	Samples Tested	No. of Samples Positive for IgA Antibodies against		
		EcLT	SB6	RTV
1	16	14	16	16
2	14	7	14	4
3	15	10	15	15
4	15	14	15	15
5	15	13	15	14
6	14	10	14	14
7	14	9	14	3
8	14	14	14	4
9	16	14	16	13
10	15	12	15	13
11	12[a]	12	12	12
12	14[a]	14	14	14
13	15	13	14	12
14	14	14	9	8
15	15	15	12	10
16	16	16	9	10
17	15	14	9	9
18	19	18	11	11
19	15	15	8	8
20	16	8	10	10
Total	299	256 (86%)	256 (86%)	215 (72%)

[a] All samples had antibodies against the three pathogens.

TABLE 2

SIgA content (g/liter) and anti-RTV IgA antibody levels in milk samples taken in the morning and in the afternoon

Subject	Age (Years)	Time Postpartum (Month)	SIgA		RTV ab[a]	
			a.m.	p.m.	a.m.	p.m.
I	19	1	0.252	0.234	2	2
W	19	2	0.897	1.003	16	16
V	26	4	0.582	0.620	2	4
X	23	5	0.303	0.350	4	4
Y	24	5	0.503	0.535	Neg	Neg
H	16	5	0.265	0.195	Neg	Neg
J	30	6	0.730	0.706	4	4
U	28	8	0.933	0.921	8	8
F	22	9	0.446	0.475	Neg	Neg
G	23	9	0.277	0.206	4	4
Mean	23.0		0.519	0.525		
SD	4.3		0.26	0.28		

[a] Reciprocal of the highest dilution giving a positive reaction. Analysis of variance: $F = 0.10749$, not significant; ($F_{0.05} = 5.12$). Neg, negative.

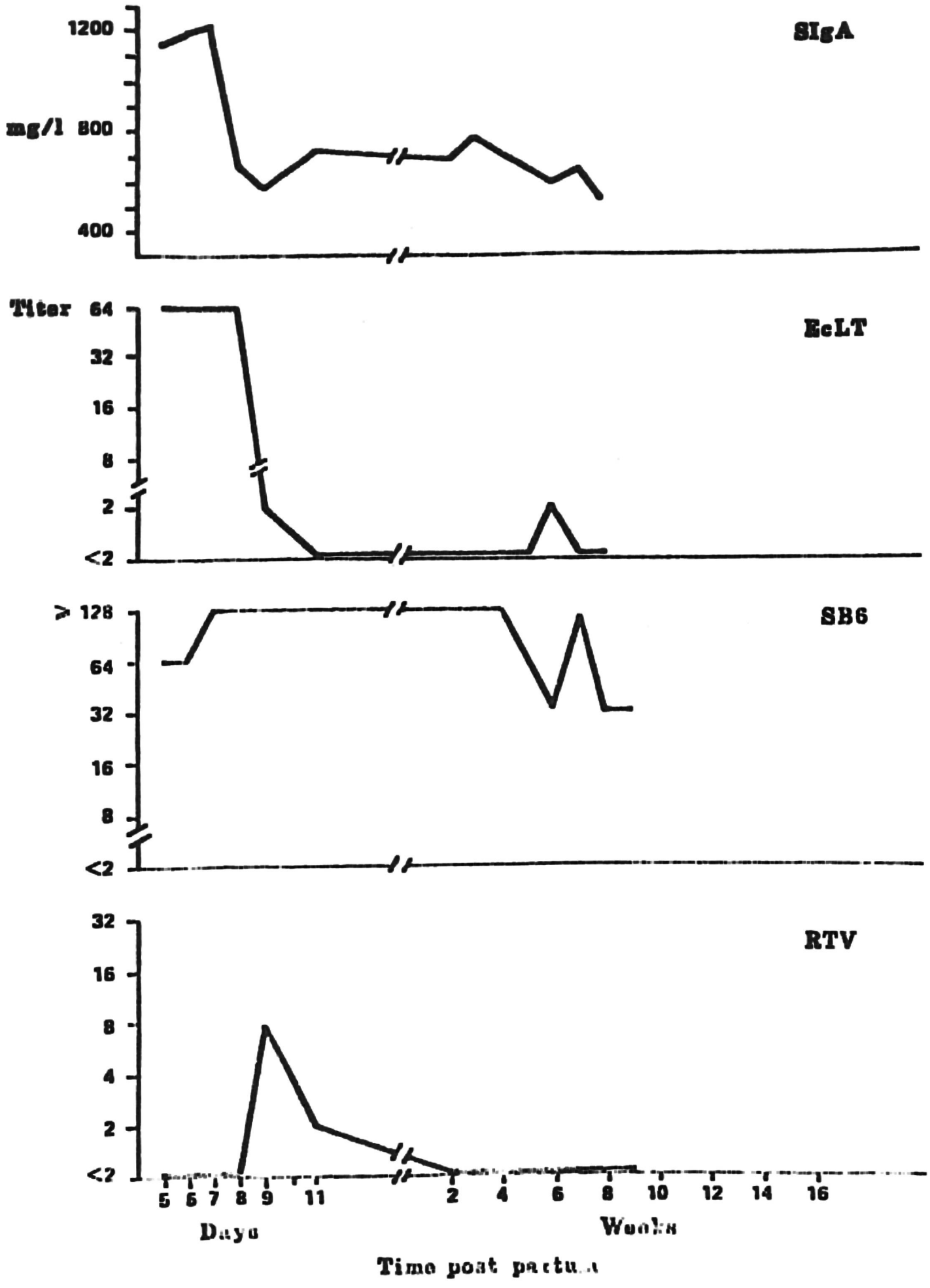

FIG. 1. Concentration of SIgA and levels of specific IgA antibodies against ECLT, SB6 and RTV in samples obtained serially from one of the mothers.

fluctuated in each of the individuals independently of total SIgA concentration and of one another. Figures 1 and 2 show the results of two representative individuals. Most important is the fact that, in 90% of the subjects, at least one of the specific antibodies reached undetectable levels[11] (Table 1). Nevertheless, all 20 mothers showed antibodies against the three pathogens in at least one of the samples obtained during the study.

The changes in concentration of milk specific antibodies do not seem to be due to sampling difficulties; this is supported by the lack of statistical interdependency between total SIgA and levels of specific antibodies.[11] Furthermore, the fluctuations of IgA antibody titers do not appear to be related to either the time of the day when the specimens were collected or to the time-relationship with an infant feeding. Using the ELISA, we examined paired samples obtained from 10 individuals in the morning and in the afternoon of the same day. The results, presented in Table 2, show that there is a difference neither in the concentration of SIgA nor in the levels of specific IgA anti-RTV antibodies in the paired samples. Similar results were obtained with milk specimens taken from

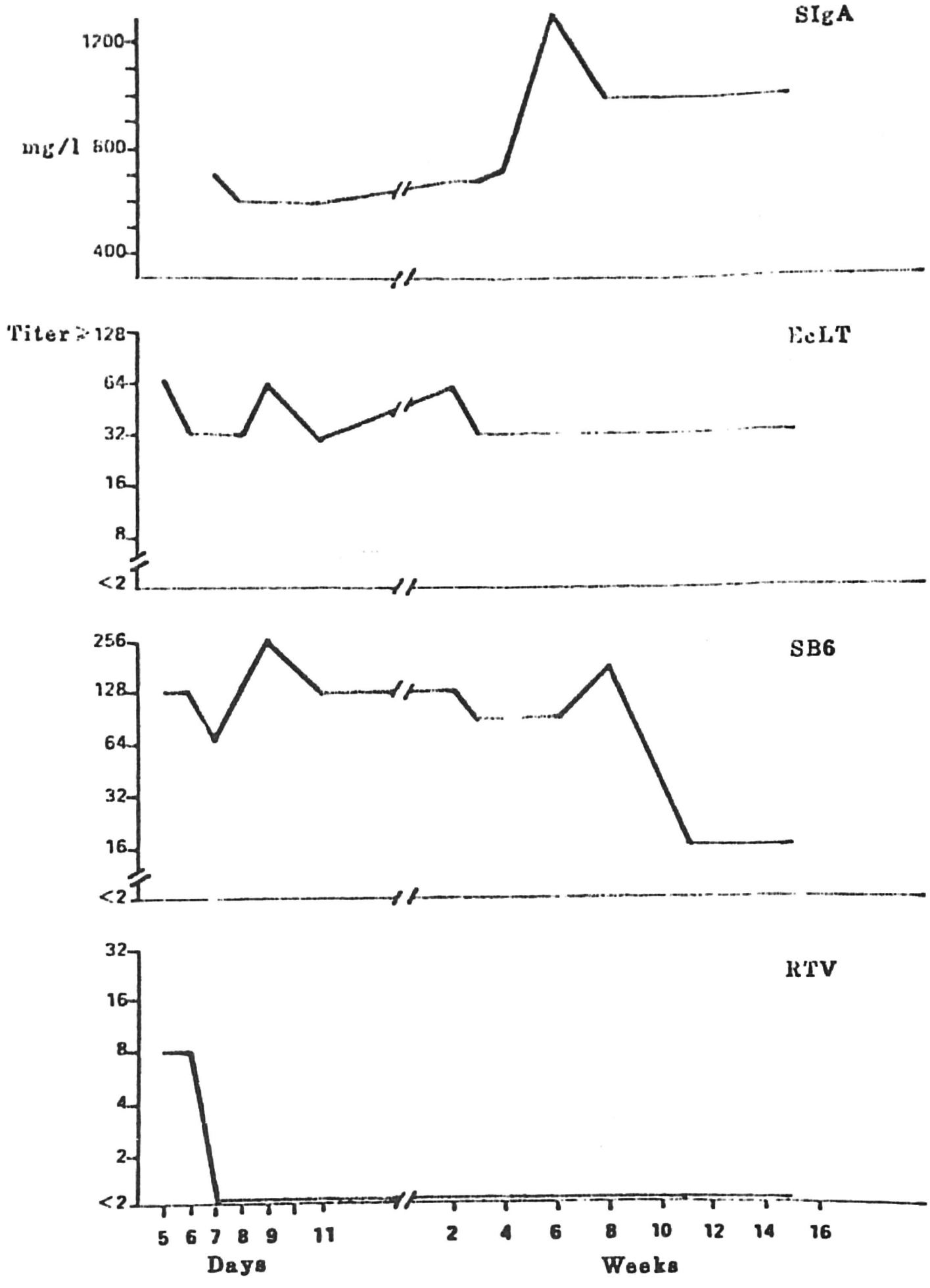

FIG. 2. Concentration of SIgA and levels of specific IgA antibodies against ECLT, SB6 and RTV in samples obtained serially from one of the mothers.

15 additional individuals, 5 minutes before and 10 minutes after a feeding (Table 3).

The fact that the levels of specific milk antibodies fluctuate and become undetectable during lactation may be related to the presence of their homologous antigen in the maternal intestine: When women who were lactopositive for antibodies against a protein extract from *Vigna sinensis* (cowpea) were given 2 g daily for 5 consecutive days of the antigen, their levels of specific anti-cowpea IgA tended to decline and reach levels which were considered negative when assayed by the ELISA (Cruz JR and Hanson LA, in press; Table 4). The fall in antibody levels was detected 1 week after initiation of the immunization protocol, and lasted 3–4 weeks. The levels of preexisting antibodies seem to play a determining role, since only those mothers with preimmunization titers above 20 responded with a sharp decline; in those with titers equal or below 20 the levels tended to increase. Hanson et al.[12] reported that vaccination of lactopositive women with live attenuated oral polio vaccine resulted in a decrease in the milk specific IgA antibodies against polio.

These observations taken together suggest that the

TABLE 3

SIgA content (g/liter) and anti-rotavirus IgA antibody levels in milk samples taken before and after a feeding

Subject	Age (Years)	Time Post-partum	SIgA		RTV ab[a]	
			Before	After	Before	After
N	15	5 d	0.647	0.720	8	8
O	25	5 d	1.102	1.262	4	4
K	26	6 d	0.535	0.540	8	8
M	26	8 d	0.657	0.762	8	8
C	27	8 d	0.573	0.625	8	8
L	26	9 d	0.550	0.432	4	4
E	23	13 d	0.376	0.327	4	4
P	19	19 d	0.625	0.595	Neg	Neg
Q	29	23 d	0.622	0.410	2	2
R	22	1 mo	0.340	0.346	4	8
T	18	1 mo	0.390	0.353	2	2
S	18	3 mo	0.220	0.215	Neg	Neg
A	29	4 mo	0.608	0.620	4	4
D	40	9 mo	0.502	0.548	8	8
B	24	12 mo	0.840	0.773	4	4
Mean	24.5		0.572	0.562		
SD	6.0		0.21	0.18		

[a] Reciprocal of the highest dilution giving positive reaction. Analysis of variance: $F = 0.26272$, not significant. ($F_{(0.05)} = 5.12$). Neg, negative.

TABLE 4

Response in human milk of lactopositive women to oral immunization with food protein from Vigna sinensis

N	Response of IgA Antibodies		
	Increase	Decrease	No change
19	2	13	4
4-fold	2	4	
8-fold		4	
≥16-fold		5	

presence of antigen in the intestine of the mother inhibits the migration of primed IgA-committed lymphocytes from the intestine to the lactating mammary gland. This inhibition of the enteromammaric transit would result in the temporary absence of specific antibodies in milk and, therefore, in decreased specific protection of the breast-fed child, in spite of continued breast-feeding. The practical implications of such phenomenon could be extremely important: if a lactopositive lactating mother becomes infected, either symptomatically or asymptomatically by an intestinal pathogen, the levels of her milk antibodies will reach very low levels. If her breast-fed child, in turn, gets infected by that given pathogen the likelihood that the infant infection becomes symptomatic (diarrhea) would be highly increased. Prospective studies on the relationship between levels of specific milk antibodies against enteric pathogens and diarrhea in the breast-fed infant are currently being undertaken by us. The role of maternal intestinal infection by pathogenic organisms in the process should also be investigated.

ACKNOWLEDGMENTS

Supported by grants from the Swedish Agency for Research Cooperation with Developing Countries, and the Diarrheal Disease Control Program of the Pan American Health Organization.

REFERENCES

1. Mata LJ: Breast-feeding: main promoter of infant health. *Am J Clin Nutr* 31:2058-2065, 1979
2. Goldman AS, Smith CW: Host resistance factors in human milk. *J Pediatr* 82:1082-1090, 1973
3. Hanson LÅ, Motas C, Barrett J, et al: Studies of the main immunoglobulin of human milk: secretory IgA. *Immunology of Reproduction*. Sofia, Bulgarian Academy of Science, pp 687-692, 1973.
4. Glass RI, Svennerholm A-M, Stoll BJ, et al: Protection against cholera in breast-fed children by antibodies in breast milk. *N Engl J Med* 308:1389-1392, 1983
5. Hanson LÅ, Ahlstedt S, Brinton CC, et al: The enteromammaric link of the secretory IgA system. *Regulation of the Immune Response*. Basel, S Karger, 1983, pp 141-153
6. Holmgren J, Hanson LÅ, Carlsson B, et al: Neutralizing antibodies against *E. coli* and *V. cholera* enterotoxins in human milk from a developing country. *Scand J Infect Dis* 5: 867-871, 1976
7. Ahlstedt S, Carlsson B, Fallström SP, et al: Antibodies in human serum and milk induced by enterobacteria and food proteins. *Immunology of the Gut* Ciba Foundation Symposium 46 (new series) Amsterdam, Elsevier/North-Holland, Inc, 1977, pp 115-134
8. Cruz JR, Garcia B, Urrutia JJ, et al: Food antibodies in milk from Guatemalan women. *J Pediatr* 99:600-602, 1981
9. Cubor G, Blacklow N, Capozza F, et al: Secretory IgA antibody to rotavirus in human milk 6-9 months postpartum *Lancet* 2:631-632, 1978
10. Goldman A, Garza C, Nichols BL, et al: Immunologic factors in human milk during the first year of lactation. *J Pediatr* 100:563-567, 1982
11. Cruz JR, Arevalo C: Fluctuation of specific IgA antibodies in human milk. *Acta Paediatr Scand*, in press, 1985
12. Hanson LA, Carlsson B, Cruz JR, et al: Immune response in the mammary gland. *Immunology of Breast Milk*. New York, Raven Press, 1979, pp 145-157