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Influence of Malnutrition on the Concentration of IgA, Lysozyme, Amylase, and Aminopeptidase in Children's Tears[1] (40024)

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Protein-calorie malnutrition (PCM) significantly increases susceptibility to infection in children (1). This may be caused by the impaired cellular immunity often observed in PCM children (2) or by suppression of humoral immunity and its associated systems, phagocytosis and complement (3). However, malnourished children often have normal or increased levels of serum immunoglobulins (4, 5). Most initial infections occur on or near mucosal surfaces which are protected from pathogens by secretory IgA, and to a lesser extent IgM, IgG, and lysozyme (6). A reduction in the relative concentration of IgA in the total protein washed from the nasopharyngeal surfaces has been reported in severely malnourished children (4). In this paper we report the effects of moderate as well as severe PCM on unmodified tears (lacrimal secretions). Concentrations of locally produced IgA, lysozyme, and amylase are compared to serum-derived components in tears.

Materials and methods. Study population. Sixty-four children of both sexes were recruited for the study in two health centers in Cali, Colombia. The children ranged in age from 2 to 5 years, with a mean of 3.75 years. All came from the same uniformly low socioeconomic background. Informed consent was obtained from the mother of each child prior to recruitment into the study. On the days that the samples were taken, each child was examined thoroughly by a pediatrician (MAR) for signs of disease (fever, diarrhea, cough, skin eruptions, etc.). Children with overt clinical signs were not sampled. The nutritional status of each child was determined by physical examination and weight-for-height using standard curves for Colombian children developed by the Instituto Colombiano de Bienestar Familiar (Colombian Institute of Family Welfare). Since such athropometric indexes are influenced by genetic differences between populations, it is important to use a standard curve which has been developed in the population from which the study sample is drawn. The ICBF weight for height scale has been found to assign a nutritional status comparable to that obtained using the widely accepted Gomez classification (7). Using this scale, Grade I children fall into 80-90%, Grade II into 70-80%, and Grade III less than 70% of the normal standard. By these criteria, 27 of the children were normal, 11 were Grade I, 9 were Grade II, and 17 were grade III malnourished.

Immunoglobulin and enzyme assays. Serum was stored at −20°. Tears were collected in 2 × 5 × 2-mm sterile sponges (Weck cel, E. Weck and Co., N.Y.) that were placed between the eyeball and the lower conjunctiva for 30-60 sec. The fluid was extracted from the wet sponge by compression into the bottom of a small polyethylene tube with a capillary pipet. A sample was taken from each eye on two different occasions 3-5 days apart. The tears were pooled and stored at 4°.

Serum IgG, IgA, IgM, and IgD were measured by radial immunodiffusion (8) using monospecific antisera (Meloy Laboratories, Springfield, Va.). IgA and IgG were quantitated in tears using commercial immunodiffusion plates for low concentrations (Hyland, Costa Mesa, Calif.). Human secretory IgA isolated from saliva (gift of Hyland Company) has a 45% lower measure level than its known concentration. Therefore the concentration of IgA obtained with the serum IgA standards was

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multiplied by 2.1 to give the concentration of dimeric secretory IgA (S-IgA). Total protein in serum and secretions was measured by the technique of Lowry *et al.* (9), modified to require only 1 μl of sample. Albumin in tears was quantitated using low-level immunodiffusion plates (Hyland). Serum albumin was measured by the method of Doumas *et al.* (10). Lysozyme (muramidase) activity in tears was measured based on hydrolysis of *Micrococcus lysodeikticus* in agar plates (11). Purified hen's egg lysozyme was used as a standard. Lysozyme values were multiplied by a factor of 2, as Bonavida *et al.* (12) have shown that the specific activity of human lysozyme is twice that of hen's egg lysozyme used as the standard. Methionine aminopeptidase activity was measured in 1 μl of tears using a modified assay for aminopeptidase in bacteria (13) by hydrolysis of 0.4 ml of 10^{-4} *M* L-methionyl β-naphthalamide in 0.1 *M* Tris-HCl buffer (pH 8.0) at 37° for 3 hr. The amount of fluorescence produced by hydrolysis of the substrate was determined with a Corning 7-60 excitation filter and Wratt 47B secondary filter. Fluorescence obtained was compared to a control solution of 10^{-4} *M* β-naphthalamide hydrolyzed/hr at 37°. Amylase activity in tears was measured by the assay of Somogyi (14). One Somogyi unit is defined as the amylase activity required for the formation of reducing power equivalent to 1 mg of glucose in 30 min at 40°.

Results. Age, sex ratio, and hemogram values. The mean ages, distribution of males and females, and hemogram results for the four groups of children are shown in Table I. The ages were similar in the four groups, although the Grade II malnourished children averaged 12% (5 months) younger than the normal children. There were nearly twice as many males as females in all groups. No evidence of anemia was observed even in the most severely malnourished children. Total and differential leukocyte counts were uniform, with somewhat elevated total counts in all malnourished children.

Immunoglobulins and enzymes in serum. Table II contains the values obtained for four immunoglobulins, total protein, albumin, and aminopeptidase in the sera of the four groups of children. Significantly elevated levels of IgA were observed in the most severely malnourished group, while concentrations of IgG, IgM, and IgD were not influenced by nutritional status. Serum albumin was significantly reduced ($P < 0.05$) in both Grade II and Grade III children, confirming the diagnosis of severe malnutrition of the Marasmus-Kwashiorkor type in these groups. Aminopeptidase was

TABLE I. AGE, SEX DISTRIBUTION, AND HEMOGRAM VALUES IN NORMAL AND MALNOURISHED CHILDREN.

	Normal	Grade I	Grade II	Grade III
Number of children	27	11	9	17
Age (months)	43 (30-57)[a]	44(27-60)	38(27-48)	45(25-60)
Sex ratio (F/M)	10/17	4/7	3/6	6/11
Hemoglobin (g%)	12.7 ± 0.8[b]	12.3 ± 0.5	12.2 ± 0.3	12.2 ± 2.8
Hematocrit (%)	39 ± 1	37 ± 1	38 ± 1	38 ± 1.9
Leukocytes (per mm^3)	10,047 ± 2123	12,190 ± 1950	11,844 ± 1333	11,580 ± 1925

[a] Mean (range).
[b] Mean ± standard error of the mean (SEM).

TABLE II. IMMUNOGLOBULINS AND OTHER SERUM PROTEINS IN NORMAL AND MALNOURISHED CHILDREN.

	Serum immunoglobulins (mg%)						
Nutritional status	IgA	IgG	IgM	IgD	Aminopeptidase[a]	Albumin (g%)	Total protein (g%)
Normal (27)	91 ± 8[b]	1089 ± 66	131 ± 11	7.5 ± 1.3	0.93 ± 0.07	4.61 ± 0.13	5.52 ± 0.25
Grade I (11)	82 ± 10	871 ± 72	97 ± 8	5.4 ± 1.6	0.98 ± 0.09	4.34 ± 0.20	5.42 ± 0.23
Grade II (9)	107 ± 18	1022 ± 107	131 ± 26	7.6 ± 2.5	0.98 ± 0.09	2.89 ± 0.21*	4.65 ± 0.36
Grade III (17)	122 ± 15*	855 ± 69	123 ± 9	7.7 ± 2.1	0.89 ± 0.10	2.70 ± 0.08*	3.85 ± 0.17*

[a] Enzyme activity expressed as units (micromoles of substrate hydrolyzed per hour at 37°).
[b] Mean ± SEM.
* Significantly different from normal children at the 5% confidence level.

found in similar concentrations in all children. The values of all serum proteins for the normal and the marginally malnourished children, Grade I, were similar.

Immunoglobulins and enzymes in tears. Table III contains the results obtained in the tears of the study children. In contrast to its elevated concentration in the serum, S-IgA was found at significantly reduced levels in the tears of Grade III children. Moderately malnourished children (Grades I and II) also tended to have lower concentrations of S-IgA and amylase than control children, although the differences were not statistically significant. Lysozyme levels and amylase activity were also significantly depressed ($P < 0.05$) in Grade III children. The values observed for total protein, albumin, and aminopeptidase in tears were unaffected by the child's nutritional status.

Linear regression analyses were performed on selected pairs of variables in tears and serum. No correlation was found between the concentration of IgA in serum and tears ($r = 0.07$, $P < 0.10$) or between levels of S-IgA and albumin in tears ($r = 0.38$, $P < 0.10$). The correlation between IgG in serum and tears was also not significant ($r = 0.16$, $P < 0.10$), while the correlation between lysozyme and S-IgA was significant ($r = 0.824$, $P < 0.05$).

Discussion. We have demonstrated reduced concentrations of S-IgA, amylase, and lysozyme in the tears of severely malnourished children. Our results in Colombia confirm the previous finding that S-IgA is decreased in the nasopharyngeal secretions of Thai and Indian children with PCM (4, 15). In addition, the synthesis or secretion of other locally produced proteins, lysozyme, and amylase into tears was impaired. Decreased activity of lysozyme has been observed in leukocytes of children with PCM (16). Reduced secretory concentrations of S-IgA and to a lesser extent, lysozyme, are potentially important factors in the defense of mucosal surfaces. The fact that total protein, IgG, aminopeptidase, and albumin in tears were not influenced by nutritional status indicates that the reduced levels of S-IgA, amylase, and lysozyme were not due to reduced volume of secretion in children with PCM, as has been suggested by experimental studies with saliva in malnourished rats (17). It appears that the mechanism involves an impairment in the local synthesis and/or secretion of these proteins.

Chandra has observed that concentrations of both S-IgA and specific antibodies to polio and measles vaccination are reduced in malnourished Indian children (15). We have confirmed these findings with respect to S-IgA in an unmodified secretion (tears) from Latin American children. Thus, it appears that malnutrition may significantly reduce both unstimulated levels of S-IgA and antibody produced to a specific antigenic challenge. These two events could have a profound influence on local immunity to an infectious agent: the former by allowing an organism to establish itself more easily on the mucosal surface and penetrate the epithelial barrier, resulting in increased infection rates; the latter by reducing the effectiveness of the immune response in dislodging an established infection, resulting in increased morbidity and prolonged recovery periods. The reduction of S-IgA, amylase, and lysozyme, which are specifically not transudated from the serum, may reflect a change in the secretory capability of the

TABLE III. AMYLASE ACTIVITY AND IMMUNOGLOBULIN LEVELS IN TEARS OF NORMAL AND MALNOURISHED CHILDREN.

Nutritional status	Amylase activity[a]	S-IgA (mg/dl/g of protein)	Lysozyme (mg/dl/g of protein)	Aminopeptidase activity[b]	Protein (g/dl)	Albumin (mg/dl)
Normal (27)	376 ± 82	49 ± 8	103 ± 18	0.58 ± 0.18	1.39 ± 0.18	34 ± 8
Grade I (11)	322 ± 52	43 ± 6	119 ± 16	0.76 ± 0.22	1.18 ± 0.16	53 ± 9
Grade II (9)	252 ± 43	31 ± 7	53 ± 9*	0.57 ± 0.16	1.63 ± 0.08	46 ± 11
Grade III (17)	199 ± 45*	27 ± 6*	62 ± 14*	0.59 ± 0.13	1.34 ± 0.17	32 ± 6

[a] Somogyi units per gram of protein ± SEM.
[b] Units (micromoles hydrolized per hour at 37°) per gram of protein.
* Significantly lower than normal children at the 5% confidence level.

mucosal surface and/or local protein synthesis. In addition, a combined reduction in immunity to some mucosal organisms such as *Neisseria gonorrhoeae* may occur since both S-IgA and lysozyme are affected. IgA antibody responses to an antigen lack the elevated secondary response memory seen in the IgG antibody response (18). Therefore, the depressed levels of S-IgA we observed in these children's secretions may represent significantly depressed secretory immunity to pathogens with which they have previously been infected as well as new pathogens infecting the mucosal surfaces. It has been shown that the S-IgA response following a single gonococcal infection which is eliminated by treatment rapidly decreases to almost undetectable levels at 4–5 months (18). Levels of S-IgA and albumin were not correlated in tears, a finding similar to that already reported in nasopharyngeal washings (4, 15). IgA synthesis in general did not seem to be impaired, since IgA was actually elevated in the serum of these same children in whose tears its concentration was suppressed. The marked contrast between elevated serum and depressed secretory IgA which we observed has been reported recently by others (4, 5). The lack of significant influence of nutritional status of IgG, IgM, and IgD concentrations in serum confirms the findings of others (4, 5). The lower levels of amylase, lysozyme, and S-IgA in tears of moderately malnourished children, although not statistically significant as in severely malnourished children, suggest that their mucosal immunity is also reduced even though they are not as nutritionally deprived.

The mucosal surfaces of the eyes and respiratory and gastrointestinal tracts serve as the portals of entry for nearly all infectious agents. Since malnourished children have increased infections of mucosal surfaces, such as gastrointestinal and ocular infections, increased antigenic stimulation of mucosal surfaces could be expected, with higher levels of S-IgA antibodies and total S-IgA rather than the lower levels we observed. The influence of renutrition on suppressed levels of S-IgA needs to be further defined as well as a clearer understanding of the mechanism of this suppression and its effects on the mucosal immune response to new antigens.

The mechanism(s) by which PCM influences S-IgA levels may be related to deficiency of dietary protein, vitamins, or trace minerals resulting in abnormal morphology or function of epithelial cells. Genetic deficiency of secretory component has been found to be associated with normal serum IgA levels with no IgA in the secretions (19). Similarly, patients with common variable hypogammaglobulinemia or selective IgA deficiency appear to have a defect in the binding of secretory component to IgA (20). We observed decreased secretory IgA concentrations in the presence of increased serum IgA levels in malnourished children, which could have resulted from impaired production and/or binding of secretory component without a reduction in the synthesis rate of IgA. Thus, increased antigenic stimulation of the mucosa due to recurrent respiratory and gastrointestinal infections in malnourished children could result in increased production of IgA which is incapable of getting through the epithelial barrier or remaining in the secretion intact.

Our observations are at variance with the elevated levels of S-IgA reported in intestinal washes from malnourished Indonesian children (21). This discrepancy is difficult to explain based upon the data available. It may be related to differences in the type of secretion samples or, more likely, to the nature of the nutritional deficiencies which accompany reduced protein and calorie intake in the two populations. It is quite possible that the presence or absence of concomitant zinc deficiency, for example, could result in the differences observed in the two studies (22). Since data on trace mineral or vitamin deficiencies are not available from either study, their relative importance remains to be evaluated.

Our findings indicate that the increased susceptibility of malnourished children to infections of mucosal surfaces may be explained, in part, by a generalized decrease in the concentrations of protective substances locally produced to protect those surfaces. Given the delicate balance which often characterizes host–parasite interac-

tions, it is not unreasonable to suggest that the decreases of 40–50% which we observed in the levels of IgA and lysozyme in the tears of malnourished subjects may be sufficient to upset the balance in favor of the pathogen.

Summary. Sixty-four pre-school-age children from a poor urban population in Colombia were studied to determine the influence of moderate to severe malnutrition on several proteins in the serum and tears. Secretory IgA, lysozyme, and amylase were found in significantly reduced concentrations in the tears of malnourished children. No influence of nutritional status on total proteins, albumin, and aminopeptidase was observed in this secretion. In the serum, IgA concentrations were significantly higher in the most malnourished group, while total proteins and albumin were reduced. These data suggest that increased susceptibility to infections of mucosal surfaces in malnourished children may be due to reduced concentrations of protective substances such as IgA and lysozyme in secretions.

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