Serum Immunoglobulins in Edematous Protein—Calorie Malnourished Children

Studies in Guatemalan Children at INCAP

JORGE ALVARADO, M.D., DAVID G. LUTHRINGER, M.D.

CLINICAL PEDIATRICS
March 1971 Vol. 10 No. 3

Copyright © 1971 by J. B. LIPPINCOTT COMPANY, Philadelphia, Pa. 19105

Serum levels of IgG, IgA and IgM were found to be either normal or elevated in PCM patients, with no relation to the degree of protein–calorie depletion, the percentage of white cells and plasmocytoid lymphocytes in peripheral blood, or the prognosis. The IgG rose in a child developing mumps even though his diet was only sufficient for nitrogen equilibrium. These results still do not elucidate the discrepancy that exists between elevated immunoglobulin fractions and the high mortality rate associated with the presence of infection in protein–calorie malnutrition.

Serum Immunoglobulins in Edematous Protein–Calorie Malnourished Children*

Studies in Guatemalan Children at INCAP

JORGE ALVARADO, M.D.,** DAVID G. LUTHRINGER, M.D.***

INCREASED susceptibility to many infections in protein–calorie malnourished children is a well-known phenomenon.[1, 2] In turn, these infections are important, not only as precipitants of kwashiorkor,[3, 4] but also as contributors to the high fatality rate in this disease.[5, 6]

The abundance of assessments of the capacity of children with protein–calorie malnutrition (PCM) to form humoral antibodies, as tested by challenge with attenuated viral and bacterial vaccines, has given variable results. For example, Brown and Katz [7] found no antibody response following yellow fever vaccine. Impaired responses to mumps vaccine and to diphtheria toxoid have been reported,[8, 9] but to typhoid and oral poliomyelitis vaccines the serologic responses have been normal.[7, 10, 11] It should be noted that these studies were done in coincidence with the giving of adequate protein therapy, and hence the responses observed may reflect the antibody-forming capacity of rapidly changing organisms during nutritional recovery rather than in static malnutrition. In PCM the rate of synthesis of gamma globulin does not seem to be impaired.[12] In fact, its synthesis is greatly increased when a superimposed infection is present, in contrast to a simultaneous decrease in the synthesis of albumin.[12] Little information is available concerning the pattern of the other serum immunoglobulins in PCM.

The investigations here described deal with the serum immunoglobulins in protein–calorie malnourished children before protein repletion was initiated. The basic question was whether immunoglobulin deficiency might be implicated in the high morbidity and mortality rates associated with infection in PCM.

Subjects

The investigations were carried out with 25 children suffering from PCM ("malnourished group") as determined by accepted clinical characteristics—poor dietary history, wasting, edema, skin and hair changes, apathy.[13]

The children were patients either in INCAP's Clinical Center or in the Pediatrics Ward of the Guatemala General Hospital. They were studied within 72 hours of ad-

* This work was sponsored in part by the National Institutes of Health of the U. S. Public Health Service (Grant No. AM-00981-12 and NIH Agreement No. 75X3903X9008-15), and by the World Health Organization.

** Assistant Chief, Biomedical Division, Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C.A.

*** Formerly, Medical Officer of the Biomedical Division, INCAP. Present Address: 1801 Eye Street, N.W., Washington, D. C. 20006.

INCAP Publication I-527.

mission, before an adequate protein intake was started. In age they ranged from 1 year and 6 months to 5 years and 11 months. Height and weight features of these children are shown in Table 1.

The percentage of expected weight for height has been selected as an index of caloric depletion, 100 per cent corresponding to the Boston 50th percentile. The mean value of all cases on admission was 89% ± 23.6 (1 S.D.), but if minimum weight is used (after clinical edema has disappeared), the mean value changes to 76% ± 3.9 (1 S.D.). This last figure was accurately obtained only in 13 children studied at INCAP's Clinical Center.

Twelve out of these 13 children were studied again by strict clinical and biochemical criteria once their nutritional recovery had been achieved. These children constitute the "recovered group."

Controls

Fourteen children served as "control group." These children came from families of low socioeconomic status and had the same age distribution as the patients. These controls, regular attenders at a day-care nursery, were selected according to the following criteria: normal growth pattern during the previous three months, absence of known clinical infection, no recent immunizations.

Methods

Venous blood samples were used for hematologic studies, including peripheral blood smears. Standard biochemical methods were used to assess nutritional status. Total serum protein was determined by refractometry.[14] Serum-protein electrophoresis was performed on cellulose acetate strips. Serum for immunoglobulin determinations was rapidly separated and kept frozen until assayed by the radial immunodiffusion technique.[15] Hyland's Immuno-Plates and immunoglobulin standards were utilized in this study (Hyland Laboratories, Los Angeles, California).

Single determinations were done on most of the samples. Random duplicates of the standards and samples gave a coefficient of variation from 5 to 15 per cent.

The patient's 24-hour urinary creatinine excretion, compared with that excreted by a normal child of the same height, independent of age, served as an index of protein depletion. This index indirectly reflects muscle mass which in itself represents approximately 43 per cent of total protein mass, and is referred to as the creatinine/height index (CHI).[16, 17]

Children suffering from PCM were treated according to our standard therapeutic regimens,[18] utilizing casein supplemented with methionine as the source of protein. A level of 0.7 Gm. of protein/Kg./day was provided for a period of 7 to 10 days, and then increased gradually to 3 or 4 Gm./Kg./day. Clinical histories were kept of recent previous infections, and detailed progress records of those that occurred during their hospitalization. Bacteriologic studies and serum immunoglobulin determinations were performed simultaneously in a few patients. Parasitologic examinations for ova and parasites were

TABLE 1. *Clinical and Biochemical Characteristics of the Malnourished, Recovered, and Control Children Studied*

Group	Age in Months	% Expected Weight for Height	% Height for Age	Total Serum Proteins	Creatinine/ Height Index
Malnourished	36 ± 15.3* (25)**	89 ± 23.6 (25)	86 ± 6.3 (25)	4.07 ± 0.53* (25)**	0.54 ± 0.15 (19)
Recovered	36 ± 12.5 (12)	104 ± 7.6 (12)	88 ± 5.1 (12)	7.40 ± 0.56 (12)	0.96 ± 0.08 (12)
Controls	45 ± 11.8 (14)	106 ± 6.5 (14)	92 ± 2.3 (14)	7.18 ± 0.64 (13)	0.96 ± 0.04 (13)

* Average ± 1 S.D.
** Number of cases.

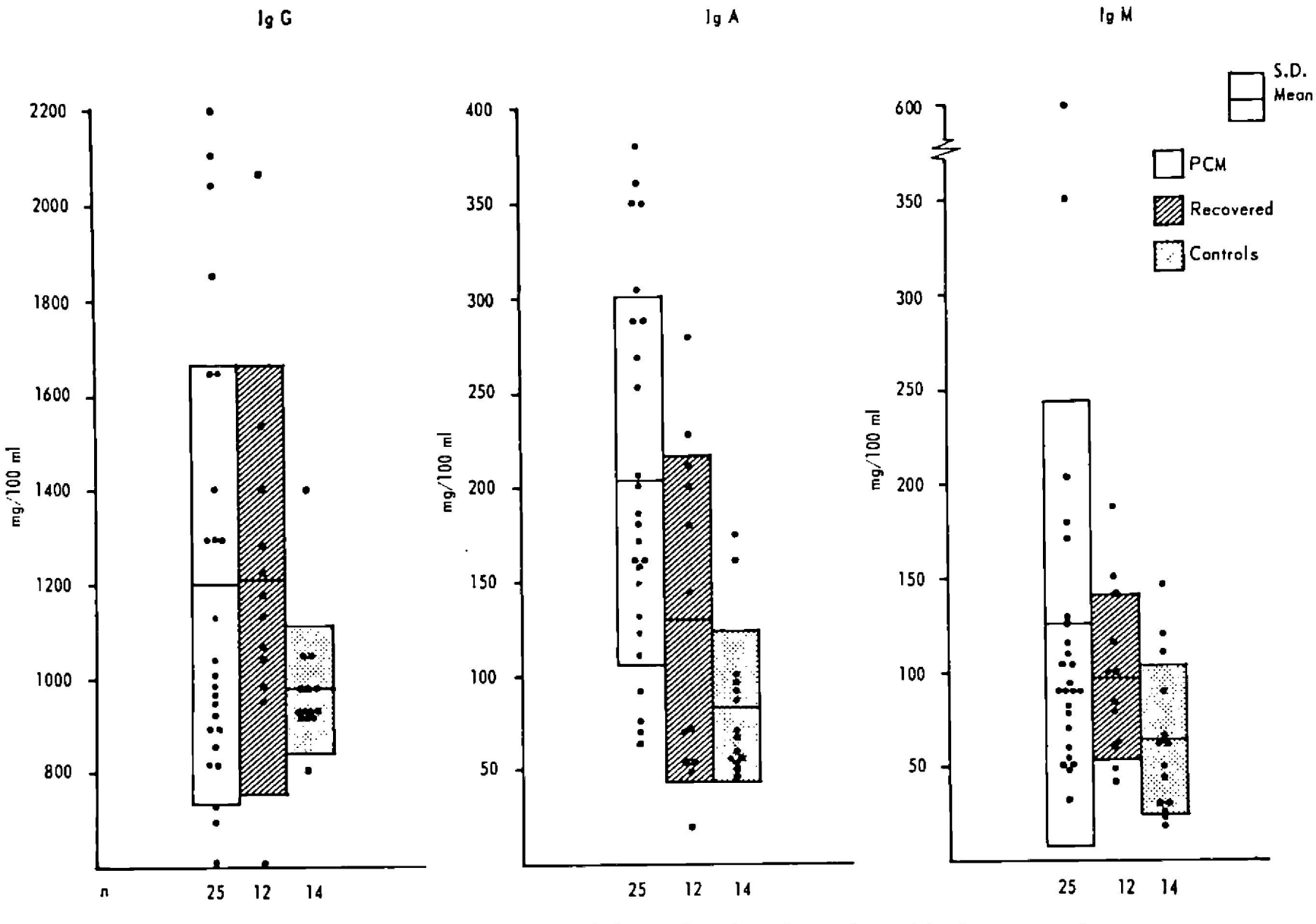

FIG. 1. Levels of serum immunoglobulin fractions in malnourished, recovered and control children.

made in stools collected from all patients. Chest x-rays were taken upon admission. After the clinical edema had disappeared (usually 10–18 days after initiation of an adequate diet) a tuberculin test was applied to all children. None of the patients showed evidence of granulomatous disease.

Results

Although indices of adequate protein nutrition are scarce and difficult to assess, two parameters were selected for this purpose (Table 2). The first parameter was the level of total serum proteins. This was found to be definitely depressed in the "malnourished group" and normal in the other two groups. The average serum albumin levels were 1.78, 4.21 and 4.06 Gm./100 ml., respectively, for the malnourished, recovered and control groups.

The second parameter was the creatinine/height index (CHI).[16, 17] Normal values in this study, as in others carried out at INCAP,[17] are always above 0.85; they usually reach 1.0 when full nutritional recovery is attained, and from then onwards remain close to this figure. The CHI in every one of our malnourished children was below 0.70 with an average value of 0.54.

No direct relation was found between the CHI (creatinine/height index) and the serum levels of immunoglobulin fractions IgG, IgA, and IgM.

No significant differences were found among the three groups—malnourished, recovered, control—with respect to IgG and IgM values (Fig. 1). Five of the 25 malnourished patients studied had IgM levels above 150 mg./100 ml., including one child (PC-210) who had 600 mg./100 ml. and was in adequate hydration when tested.

Patients with severe diarrhea had the highest values of both IgM and IgA serum immunoglobulins.

Between the malnourished group and the normal controls a significant statistical differ-

TABLE 2. *Serum Immunoglobulins in the Groups of Children Studied*

Group	Immunoglobulins		
	IgG	IgA	IgM
Malnourished	1,201 ± 465 (25)	203 ± 98 (25)*†	126 ± 118 (25)
Recovered	1,210 ± 456 (12)	130 ± 87 (12)*	97 ± 44 (12)
Controls	977 ± 136 (14)	83 ± 40 (14)†	64 ± 30 (14)

* $p < 0.05$ within groups.
† $p < 0.001$ within groups.

ence in regard to IgA levels ($p < 0.001$) was observed. The differences in levels between malnourished and recovered children was also significant ($p < 0.05$). No significant difference in levels was observed between recovered and normal children. Figure 2 compares the IgA values for these Guatemalan children with values reported for healthy Scandinavian children.[19]

When the children with PCM were divided into two groups according to their age: below or above 36 months, the mean serum immunoglobulin fractions of the two groups did not differ significantly. Age, therefore, does not explain the results obtained. The values for the 86 per cent of the normal control children in our study fall within 2 S.D. of the mean values reported for normal Scandinavian children [19] (Fig. 2).

Three cases were studied serially (Table 3). Throughout, patient 196 had a constant increase in the IgA fraction. The last determination, done one week after a lactose load test of four days duration, demonstrated lactose intolerance. Patient 198 maintained high levels of IgA throughout the first 62 days of hospitalization and had severe diarrhea during the first week after admission even when given a lactose- and gluten-free diet. Child 216 was studied at more frequent intervals during the first two weeks of hospitalization; parotitis was obvious on the fifth day, and the IgG level rose rapidly even while dietary protein was given in amounts which only allow nitrogen equilibrium. Clinically, this patient behaved as an otherwise normal child, had high fever four days after the onset of parotitis and developed no complications.

Three of our malnourished patients had a fatal course. Patient 183 died as a consequence of electrolyte imbalance and severe lactic acidosis eight days after admission; pa-

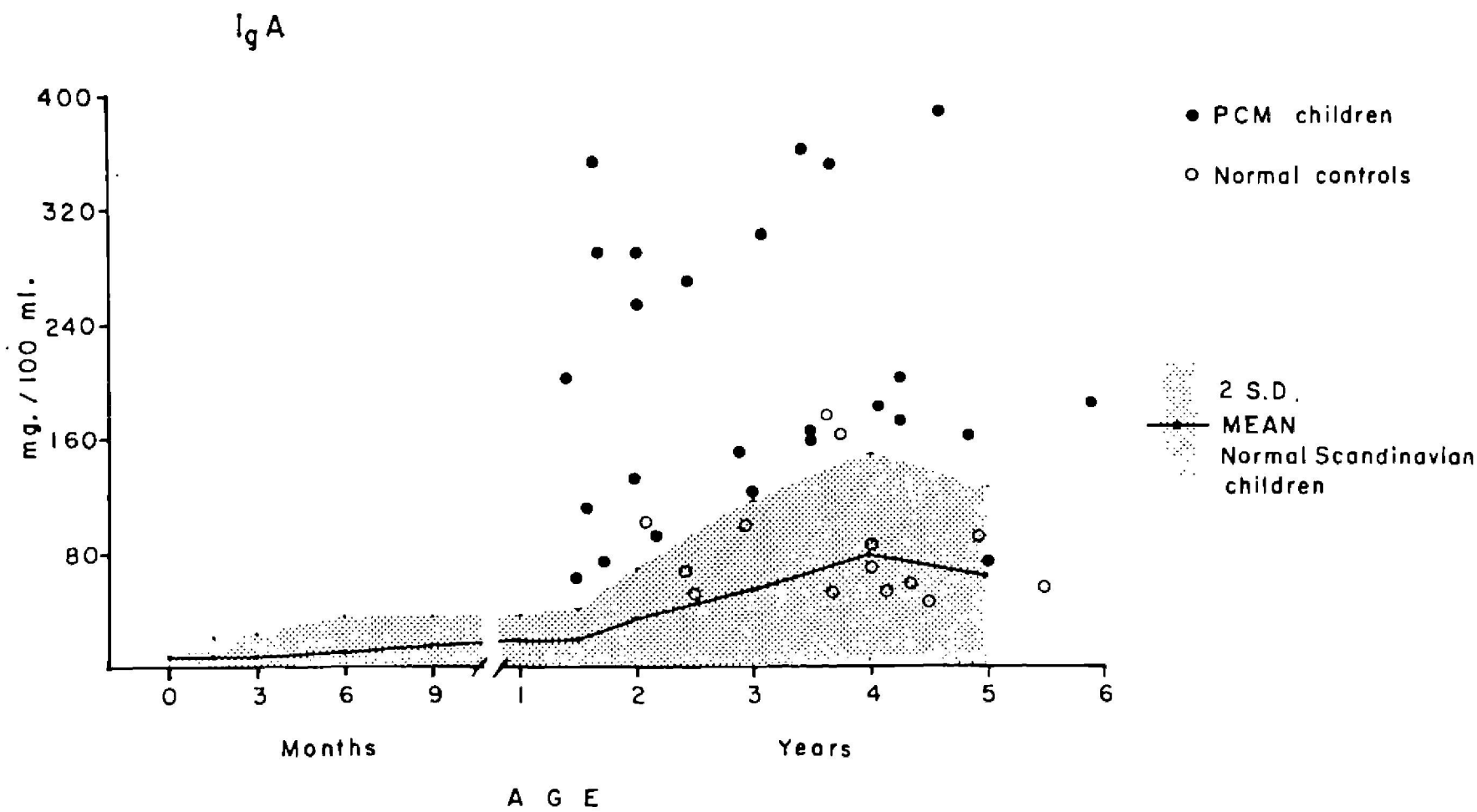

FIG. 2. Serum IgA levels in protein-calorie malnourished children and normal Guatemalan controls, compared with healthy Scandinavian children. (Adapted from Johansson, D. O. and Berg, T.: Acta Paed. Scand. 56: 572–579, 1967.)

TABLE 3. *Longitudinal Studies*

Code	Days of Hospital Stay	Diarrhea	Respiratory Infection	Other Diseases	Total Serum Proteins (Gm.%)	Serum Albumin (Gm.%)	Immunoglobulins IgG (mg.%)	IgA (mg.%)	IgM (mg.%)
196	1	+	0	Herpes simplex	3.3	1.53	820	148	78
	28	0	0	0	7.1	4.04	1,050	122	140
	65	0	+	Fever	5.1	3.04	1,350	215	58
	120	Lactose intolerance	0	0	6.9	4.60	1,175	200	41
198	1	++	+	0	3.6	1.73	2,050	360	65
	45	0	0	0	7.9	4.94	1,400	173	120
	62	0	0	0	8.1	5.36	980	280	100
216	1	++	0	Conjunctivitis	3.1	1.32	890	171	136
	3	+	0	Conjunctivitis	3.2	1.40	890	160	104
	8	+	0	Mumps	4.5	1.64	1,450	304	180
	18	0	0	0	6.4	3.17	1,530	163	166

tient 184 died without 48 hours from severe bronchopneumonia, and patient 213 died after 12 days of hospitalization from severe generalized septicemia caused by *Pseudomonas aeruginosa*. None of these fatal cases had subnormal levels in any of the immunoglobulin fractions that were measured.

Total white blood cell and differential counts in 14 of the malnourished children, done upon admission and simultaneously with the chemistry studies, showed no positive correlations between the percentage of any of the white cells, including the abnormal circulating plasmocytoid lymphocytes and the three immunoglobulin fractions studied.

In bacteriologic investigations of those patients with severe diarrhea or with suspected generalized sepsis, *Salmonella* was found in the stools of two with severe gastrointestinal symptoms, and *Pseudomona aeruginosa* grew in culture samples taken from the skin, blood and feces of patient 213. No relation was found between the highest rectal temperature observed during the first week of admission and the level of any of the Ig fractions. Of interest, however, is that none of the patients had severe hypothermia (a common sign in septicemia).

Intestinal parasites, when present, were usually the combination of helminths and protozoa. Neither severe infections nor a significant relation between the immunoglobulin levels and the parasitic load were observed.

Discussion

With rare exceptions, children with PCM are either incubating or harboring clinical or subclinical infections of varied etiology. This would explain the stimulation for an increased rate of synthesis of serum immunoglobulin in some PCM patients, as observed by Cohen and Hansen,[12] and ourselves. Furthermore, these rises suggest that the high incidence of infection associated with PCM is not a consequence of immunologic deficiency as measured by levels of IgG, IgA and IgM in serum.

The significantly higher-than-normal levels of serum IgA observed in this and other series of malnourished children, as recently reported,[8, 20, 21] have not yet been adequately explained. The elevations in some of the malabsorptive and the diarrheal disorders,[22] could be related to repetitive gastrointestinal insults, which are of common occurrence in children with PCM. An abnormal permeability of the gastrointestinal mucosa, if present, could facilitate the entry into the blood stream of the IgA secreted by the plasma cells in the *lamina propria*. Immunofluorescence studies of biopsies of gastrointestinal mucosa in celiac disease have revealed an increased number of plasma cells infiltrating the *lamina propria*, the majority of them staining with antisera to IgA and a few with antisera to IgG and IgM.[23] Morphologic studies of duo-

denal and rectal biopsies in PCM children have shown lymphoid and plasma cell infiltrations of the *lamina propria,* which do not entirely disappear with nutritional recovery.[24] If these plasma cells are producing IgA, as is the case in celiac disease,[25] this may help explain the rise of serum IgA levels in PCM. The persistence of the high IgA levels after nutritional recovery, could similarly reflect what is occurring in the intestinal mucosa.

In a recently reported study of serum immunoglobulin levels [21] in malnourished children, the control group consisted of children from rural communities where PCM and intermittent diarrhea are prevalent. These subjects had neither clinical nor biochemical evidence of malnutrition, but their serum IgA values were similar to those of the "recovered" patients in our present study and differ significantly from values in children with PCM.

Acknowledgments

The authors express their sincere appreciation to Mr. José Luis Escobar, for his valuable technical assistance, to Mrs. Ilse de Melgar, for her assistance in the statistical analysis of the data, and to Dr. Victor Argueta von Kaenel, Head of the Pediatric Department of the Guatemala General Hospital, for allowing us to study patients under his care. They also gratefully acknowledge the encouragement received from Dr. Fernando Viteri, Head of the Biomedical Division of INCAP, and from Dr. Moisés Béhar, Director of the Institute.

References

1. Gordon, J. E., Chitkara, I. D. and Wyon, J. B.: Weanling diarrhea. Amer. J. Med. Sci. 245: 345, 1963.
2. Scrimshaw, N. S., Wilson, D. and Bressani, R.: Infection and kwashiorkor. J. Trop. Pediat. 6: 37, 1960.
3. Scrimshaw, N. S., Taylor, C. E. and Gordon, J. E.: Interactions of nutrition and infection. Geneva, World Health Organization, 1968 (WHO Monograph Series No. 57), p. 26.
4. Salomon, J. B., Mata, L. J. and Gordon, J. E.: Malnutrition and the common communicable diseases of childhood in rural Guatemala. Amer. J. Pub. Health 58: 505, 1968.
5. Tejada V., C., Béhar, M. y Cofiño U., E.: Estudio clínico patológico de las bronconeumonías del niño desnutrido. Rev. Col. Méd. (Guatemala) 7: 134, 1956.
6. Lawless, J., Lawless, M. M. and Garden, A. S.: Admission and mortality in a children's ward in an urban tropical hospital. Lancet 2: 1175, 1966.
7. Brown, R. E. and Katz, M.: Antigenic stimulation in undernourished children. E. Afr. Med. J. 42: 221, 1965.
8. Kumate R., J.: Desnutrición e inmunologia. *In* Desnutrición en el Niño, R. Ramos Galván, C. Mariscal Abascal, A. Viniegra Carreras and B. Pérez Ortiz, Eds. México, D. F., Impresiones Modernas, S. A., 1969; p. 121.
9. Olarte, J., Cravioto, J. and Campos, B.: Inmunidad en el niño desnutrido. I. Producción de antitoxina diftérica. Bol. Méd. Hosp. Infant. (Méx.) 13: 467, 1956.
10. Pretorius, P. J. and De Villiers, L. S.: Antibody response in children with protein malnutrition. Amer. J. Clin. Nutr. 10: 379, 1962.
11. Kumate, J., Benavides, L., Hikimura, J. y Herrera Romo, L.: Respuesta inmunológica en fiebre tifoidea. Bol. Méd. Hosp. Infant. (Méx.) 19: 17, 1962.
12. Cohen, S. and Hansen, J. D. L.: Metabolism of albumin and γ-globulin in kwashiorkor. Clin. Sci. 23: 351, 1962.
13. Viteri, F., Béhar, M., Arroyave, G. and Scrimshaw, N. S.: Clinical aspects of protein malnutrition. *In* Mammalian Protein Metabolism, H. N. Munro and J. B. Allison, Eds. vol. 2, New York, Academic Press, Inc., 1964; pp. 523–568.
14. Instructions for use and care of T. S. meter and the T. C. Refractometer. A. O. Instrument Company, Buffalo, New York 14215, U. S. A.
15. Fahey, J. L. and McKelvey, E. M.: Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immun. 94: 84, 1965.
16. Viteri, F., Alvarado, J., Luthringer, D. G. and Wood, R. P. III.: Hematological changes in protein–calorie malnutrition. Vitamins Hormones 26: 573, 1968.
17. Viteri, F. and Alvarado, J.: The creatinine height index; its use in the quantification of protein depletion and repletion in protein–calorie malnourished children. Pediatrics. (In press)
18. Alvarado, J., Viteri, F. and Béhar, M.: Tratamiento hospitalario de la desnutrición proteínico–carórica severa. Rev. Col. Méd. (Guatemala), 1970. (In press)
19. Johansson, D. O. and Berg, T.: Immunoglobulin levels in healthy children. Acta. Paediat. Scand. 56: 572, 1967.
20. Keet, M. P. and Thom, H.: Serum immunoglobulins in kwashiorkor. Arch. Dis. Child. 44: 600, 1969.
21. Lechtig, A., Arroyave, G., Viteri, F. and Mata, L. J.: Inmunoglobulinas séricas en la desnutrición proteínico–calórica de niños preescolares. Arch. Latinoamer. Nutr., 1970. (In press)
22. Najjar, S. S., Stephan, M. and Asfour, R. Y.: Serum levels of immunoglobulins in marasmic infants. Arch. Dis. Child. 44: 120, 1969.
23. Rubin, W., Fauci, A. S., Sleisenger, M. H. and Jeffries, G. H.: Immunofluorescent studies in adult celiac disease. J. Clin. Invest. 44: 475, 1965.
24. Schneider, R., Alvarado, J. and Viteri, F.: Histopathological study of the intestinal mucosa in malnutrition: A preliminary report. *In:* Pediatrics in Latin America. Report of a Macy Conference, Santiago, Chile, March 24 to 26, 1969. Josiah Macy Jr. Foundation, p. 34.
25. Thompson, R. A., Asquith, P. and Cooke, W. T.: Secretory IgA in the serum. Lancet 2: 517, 1969.