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SERUM ANTIBODY RESPONSE OF MALNOURISHED CHILDREN AS COMPARED WITH WELL NOURISHED CHILDREN

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There is a great deal of clinical and experimental evidence to support a close relationship between nutritional status and the severity of many infections. In general terms, bacterial, rickettsial and helminth infections become more severe in the presence of malnutrition and the effect on viruses and protozoa varies with the species of deficiency.[1]

There is evidence in current literature of a relationship between severe hypoproteinemia and decreased antibody response in animals. Numerous studies with dogs,[2,3] rabbits,[4,5] rats[6,7] and mice[8] indicate clearly that under conditions of severe protein deficiency, antibody formation may be adversely affected.

The information in humans is limited to a few studies and the results are conflicting and inconclusive. Wohl, Reinhold and Rose[9] demonstrated that patients with disturbance of protein metabolism sufficient to cause low serum albumin show an appreciable impairment of antibody response to typhoid vaccine. Total absence of antibody response to typhoid vaccine in children with kwashiorkor was also found by Budiansky and Neves Da Silva in Brazil.[10] Olarte, Cravioto and Campos[11] in Mexico demonstrated an appreciably decreased antibody response against diphtheria toxoid in children with low serum proteins.

The effect was noticed mainly in two subjects with total proteins of 4.2 and 3.8 gm. % respectively.

Some other attempts to demonstrate this in man[12,13] have failed, presumably due to the relatively mild protein malnutrition in subjects studied. The conclusion from these latter studies of malnutrition and antibody formation in man is not that he differs from other animals in this respect, but that sufficiently severe protein deficiencies in man are not readily produced by feeding deficient diets to well nourished individuals for relatively short periods, nor even by the type of depletion usually associated with chronic disease.[14]

The objective of this study is to determine whether severe protein deficiency with or without caloric deficiency of comparable severity will interfere with the production of serum antibodies

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against typhoid resulting from the administration of typhoid-paratyphoid vaccine. In so doing, the antibody response of well nourished urban children, rural Mayan Indian children and children hospitalized with kwashiorkor or marasmus were compared.

MATERIAL AND METHODS

Observations were made in thirty-three children of both sexes, from 2 to 9 yrs of age. Thirteen were malnourished children hospitalized at the Guatemala City General Hospital. Of these, ten had kwashiorkor and three had marasmus. During the period of observation, these children were given a therapeutic hospital diet high in milk and other protein of animal origin. The fact that this high intake of protein of good biological value could have an effect on the formation of antibodies was recognized, but due to the severe nature of the disease it was considered ethically impossible to deprive these children of dietary treatment. Nevertheless, a decreased response was considered probable due to the chronic malnutrition present at the time of the initial dose of vaccine.

A second group, which served as control, consisted of ten well nourished and well developed children from well-to-do families in Guatemala City.

The remaining ten children were selected from a rural Guatemalan village, Santiago Sacatepéquez. This predominantly Mayan Indian community of about 4,000 inhabitants is located in the Guatemalan highlands approximately 20 miles west of Guatemala City. Maize in the form of tortillas is the principal dietary staple and about one pound of maize per person per day is consumed in this manner. Other cereal foods are very rarely used and milk is practically absent from the diet. The average per capita consumption of meat is 1/3 lb per day. Many families raise chickens, but eggs are rarely eaten. The diet is based on maize, and a small variety of vegetables (squash, red tomato). Although the total dietary protein is adequate in amount for most adults, children often do not receive their proportionate share. Furthermore, that of animal origin is always less than a fourth of the total.[15] The children, although lacking specific nutritional deficiency signs, were shorter on the average, and weighed less for their age than the control group.

Children with a past history of typhoid fever were excluded. Children with previous typhoid vaccination were also not knowingly selected, although many parents were unable to recall whether or not their child had been vaccinated against typhoid fever.

Height, weight, and various physical signs and symptoms of possible relation to malnutrition were recorded on special cards at the beginning of the study. Each child received three doses of 0.5 ml of typhoid and paratyphoid vaccine (Lederle) subcutaneously at intervals of one week.

In the malnourished group, a specimen of one cubic centimeter of blood from the fingertip was obtained before the first injection and at weekly intervals until 3 weeks after the third dose. In the control and village groups, blood specimens were taken only before the first and third dose of vaccine and two weeks after the third dose. The serum was obtained by centrifuging of the blood sample. Total proteins were determined within 4 hours by the densitometric method of Lowry and Hunter.[16] Serum albumin was determined according to the method described by Debro et al.[17] and globulins were calculated by difference.

Antibody titers against O and H antigens (Lederle's, Salmonella Group D Antigen, Typhoid O, Somatic IX, XII; and Typhoid H Antigen, flagelar d) were determined simultaneously in all sera collected after all samples were taken. Slide test for serum agglutinins was utilized. The serum to be tested was pipetted in amounts of 0.08, 0.04, 0.02, 0.01, 0.005 and 0.002 ml with a Kahn pipette into each of the rings of the glass slide, starting with the greatest amount. All antigens were shaken before using. One drop (0.03 ml) of the antigen was added to each of the serum amounts. Each ring of the serum antigen mixture was mixed thoroughly for 15 seconds with the end of a wooden applicator stick starting with the smallest amount of serum; after mixing, the glass slide was gently rotated 20 to 30 times throughout the 3 min. reaction time following the addition of the antigen and the drops were carefully watched for clumping. The degree of agglutination was then estimated at once by three persons who recorded their results separately. Origin of serum was unknown at the time of the test. Agglutination of 50% or more was considered as the "end point of serum reactivity" or as the serum titer. Dilutions of 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640 were used. Antigen controls made with known positive and negative sera were tested at least twice a day.

Scheme of Injections and Blood Specimens

	Weeks 0	1	2	3	4	5
Malnourished	B-V_1	B-V_2	B-V_3	B	B	B
Well nourished (Control)	B-V_1	V_2	B-V_3	-	B	-
Rural village	B-V_1	V_2	B-V_3	-	B	-

V - vaccine B - Blood sample

RESULTS

A) Antibody Response:

The antibody response at 14 and 28 days after the first dose of vaccine was compared and evaluated by the Chi-square test. No significant differences were found among the 4 groups at the various serum dilutions employed. The mean and standard devia-

Table 1. Weekly Average of Agglutination Titers in the Groups Studied.

Groups	No. of Cases	Antigen	DAYS 0	7	14	21	28
Kwashiorkor	10	H	4	56	190	262	269
Marasmus	3	H	7	7	40	107	93
Control	10	H	0	-	132	-	212
Village	10	H	4	-	140	-	200
Kwashiorkor	10	O	4	82	76	78	67
Marasmus	3	O	13	53	53	53	53
Control	10	O	4	-	108	-	82
Village	10	O	4	-	62	-	28

Table 2. Weekly Average of Titers (Excluding Cases which Showed Agglutination Before the First Dose of Vaccine.)

Groups	No. of Cases	Antigen	DAYS 0	7	14	21	28
Kwashiorkor	9	H	0	58	193	220	222
Marasmus	2	H	0	0	40	120	120
Control	10	H	0	-	132	-	212
Village	9	H	0	-	107	-	151
Kwashiorkor	9	O	0	73	67	69	65
Marasmus	2	O	0	40	40	60	60
Control	9	O	0	-	111	-	73
Village	9	O	0	-	60	-	22

tion were not considered proper measures for summarizing the results due to the fact that serial twofold dilutions were used instead of a continuous scale. Nevertheless, weekly averages of agglutination titers against H and O antigens were calculated in the four groups for the purpose of comparison. These calculations are summarized in Tables 1 and 2. Even though there were no statistically significant differences among the various groups, the following observations were made which may serve as leads for future investigations.

The antibody response to H antigen is similar in both tables. The average titers are not significantly affected by the exclusion of the cases which showed agglutination before the first dose of vaccine. In general, there is a higher response shown at 14 and 28 days by the kwashiorkor cases. On the other hand, the average titers observed in the marasmus cases are considerably lower than the corresponding titers in the other 3 groups. Excluding secondary responses, the village group shows a somewhat lower response than the control.

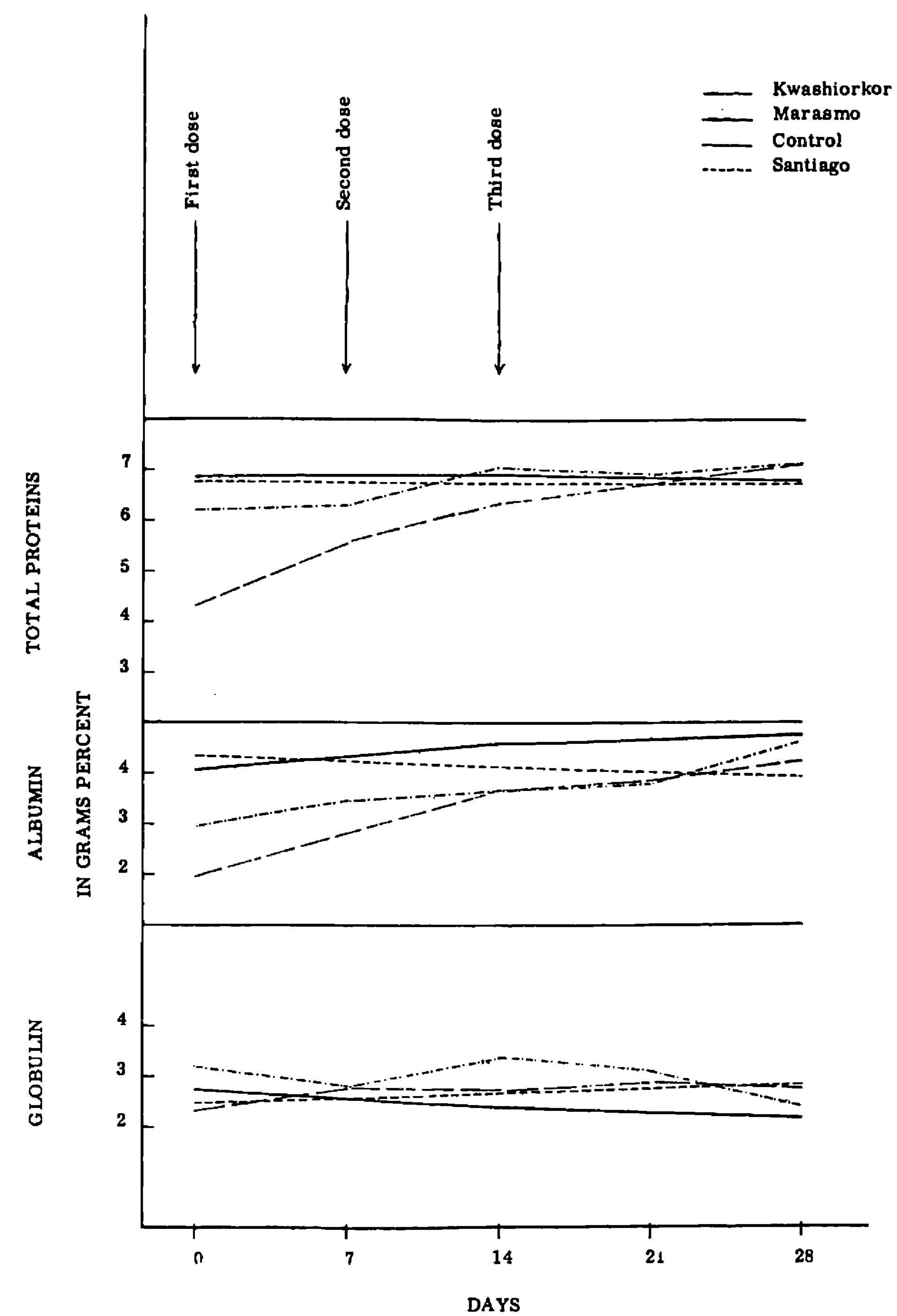
The 0 antibody titer was less pronounced and too variable to warrant detailed discussion. There was, however, a rise in titer at 14 days in all groups, followed by a drop at 28 days, except in the three marasmus cases; these maintained the same or similar titers from 7 to 28 days. Again the village groups show a less marked response at 14 days and the response was even lower at 28 days.

B) Blood Protein Changes (Fig. 1)

Variations in the average total protein levels occurred as expected. Beginning with very low values, there was a rapid weekly rise in the children with kwashiorkor. On the average, these children attained normal levels after two weeks of observation and reached values similar to those of the control and village groups at 21 and 28 days. The marasmus cases showed normal levels from the beginning of the study followed by a slow steady rise. The serum protein concentrations in the control and village groups remained almost the same with very slight variations during the period of observation.

A continuous rise in serum albumin concentration was also observed in the malnourished children. The rise was more pronounced in the cases with kwashiorkor showing an increase from an average of 1.97 g % at the beginning of the study to an average of 3.68 and 3.88 respectively two and three weeks later. Once more, the variations observed in the control and village groups were not significant. Nevertheless, there was a slight continuous rise

SERUM PROTEIN RESPONSE
Fig. 1



in the control group compared with a slow fall in the village children.

A rise in average serum globulin for the 3 children with marasmus occurred at the same time of the third dose of vaccine. This increase coincided with the rise in H antibody titer.

A similar serum globulin increase was not observed in the cases with kwashiorkor in spite of the fact that a rise in titer was also observed at this time. No significant change in globulin concentration occurred in either the village or control group, although there was a slight downward trend in the serum globulin of the control group despite an increase in the antibody titers.

DISCUSSION

Since the antibody titer results obtained in this study were not significantly different among the four groups, no direct relation was demonstrated between the capacity to produce antibodies and nutritional status. Moreover, the initial severity of malnutrition showed no direct relation to the production of antibodies. For example, a number of well nourished children showed a poor antibody response while some cases of kwashiorkor had a surprisingly high response. Nevertheless, the better response obtained by kwashiorkor children 14 and 28 days after the first dose may have been due to the fact that these children had a greatly increased nitrogen retention.[18] Since they were receiving foods containing an abundance of good quality protein from the beginning of the study, it is possible that the necessary amino acids were incorporated into the body's protein pool very early in treatment and thus were available for the protein requirements of the organism, particularly for the formation of antibodies. This explanation seems plausible since a favorable effect of protein supplementation on antibody formation has been previously demonstrated in chronically ill adults.[9] Similarly, children receiving high protein diets may be more likely to develop prompt and pronounced antibody responses despite their poor nutritional status at the time the first dose of vaccine is given.

Since only three cases were studied, the lower response observed in marasmus must be interpreted with caution. Nevertheless in all the cases the decreased response was quite marked. Although marasmus cases in general are slower to respond clinically to diet therapy, it is difficult to understand why a high protein diet should not be at least as effective in supporting antibody synthesis in marasmus as in kwashiorkor. If further study should confirm this finding, it will be one more example of clear cut metabolic differences between the two conditions, although **at priori** the difference in this case might have been expected in the reverse direction. At least, other biochemical processes have been shown to be more seriously affected in kwashiorkor than in marasmus.[19]

Although the response obtained in the village group was slightly lower than the control group, it is of great importance from the public health point of view that the antibody response of the two groups was not significantly different. This provides evidence in support of future vaccination programs against typhoid and paratyphoid infections in the rural areas of Guatemala, even among malnourished individuals. Whether the lower response in the village groups can be attributed only to random variation or whether constitutional, racial or nutritional factors are also involved is a matter that needs further study.

The variations observed in serum protein levels were not unexpected. The rapid recovery and prompt achievement of normal protein values and serum albumin levels observed in the kwashiorkor cases is in agreement with previous findings.[20]

There was no appreciable increase in serum globulin which could be related to a rise of antibody titers. Rapidly increasing levels were observed in 4 kwashiorkor cases and in every case they were related to an intercurrent infection (U.R.I., varicella, parotiditis). These data are in agreement with the fact that agglutination titers against H and O antigens, although they detect very small amounts of antibody, are not always specific and fail to give an indication of the absolute quantity of circulating immune globulin produced. Thus, it is possible that the serum containing but little specific antibody may possess a relatively high agglutination titer.[21,22]

The data suggest that a continuing severe state of malnutrition is necessary in order to significantly alter the formation of antibodies. In this study protein repletion may have been affecting antibody formation in a favorable manner. In the absence of diarrhea, or any other condition that may prevent or decrease protein absorption from the intestinal tract, dietary protein is readily made available to satisfy the needs of the organism. It seems likely that high protein diets may play an important role in increasing resistance to infection in malnourished children through the mechanism of acquired immunity.

SUMMARY

1. Thirty-three children were vaccinated with T.A.B. vaccine to determine their ability to produce antibodies against H and O typhoid antigens. These children were distributed in 4 groups: 10 well nourished, 10 kwashiorkor, 3 marasmus and 10 selected from a rural Guatemalan village.

2. The children with kwashiorkor were receiving a therapeutic diet high in protein during the period of observation. Under these circumstances, they proved as capable of producing

antibodies as well as, or better than, the well nourished controls.

3. A low antibody response was observed in 3 marasmus cases studied.

4. Village children were found to have a satisfactory response, although slightly lower than the controls. We stress the importance of these findings in public health vaccination programs.

5. None of the differences found among the 4 groups at the various serum dilutions employed, were statistically significant.

6. No relation was found between antibody response, total serum protein, serum albumin or serum globulin levels.

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