

## Complement and C3 Proactivator Levels in Children with Protein—Calorie Malnutrition and the Effect of Dietary Treatment

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**INTRODUCTION:** It is generally stated that malnutrition is associated with increased susceptibility to infection and that, in malnourished individuals, the infection is often more severe and recovery is slower than in well-nourished persons. Although association between protein—calorie malnutrition (PCM) and the increased susceptibility to infection has never been adequately proven by carefully controlled clinical studies, the weight of clinical experience by many investigators suggests that such a relationship does indeed exist. On the other hand, slow recovery from infection in PCM has been adequately documented, especially in experimental animals. It is difficult to design and control experiments in humans showing that malnourished individuals are more susceptible to infection; one can, however, look for changes in components known to be associated with defense mechanisms against infection, e.g., cell—mediated immune function, antibody and inflammatory responses, phagocytic and microbicidal activities of leukocytes, and the complement system.

The effect of PCM on the humoral immune response has been extensively investigated; the results are, however, inconclusive. While antibody response to some antigens was markedly depressed in PCM, the response to most antigens was unaffected. On the other hand, the cell—mediated immune function in a malnourished population is depressed somewhat. The hemolytic complement activity of the serum from malnourished children is also less than that of the well—fed children. Because a defect of the complement system is known to be associated with increased susceptibility to infection and malnourished children are prone to develop a gram—negative septicemia, impairment of the complement system in these children might be expected. The present study was therefore designed to examine the effect of protein—calorie malnutrition on both the classical complement system and the newly discovered alternate C3—activating system. The serum levels of individual complement proteins, including C1q, C1s, C3, C4, C5, C6, C8 and C9, and of C3—proactivator (C3PA) of children with kwashiorkor and marasmus were determined on hospital admission and at intervals thereafter during dietary treatment. The results showed that, although the admission levels of these complement components and of C3PA were significantly lower than those of well—nourished children of the same age, most components rose to "normal" levels after appropriate dietary treatment.

### MATERIALS AND METHODS:

**Patients:** The patients, age 1 to 5 years, were admitted to the research ward of the Anemia and Malnutrition Research Center in Chiangmai, Thailand, and remained in the hospital throughout the study period. On admission, the patients were clinically evaluated and classified as having marasmus or kwashiorkor according to the clinical criteria. Only children with primary malnutrition who weighed more than 3 kilograms but less than 12 kilograms were admitted to the study. All patients were evaluated on admission for evidence of infection, using clinical impression and the results of differential leukocyte counts, chest X—ray, and

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blood, urine and stool cultures. By these criteria, a majority of the 20 malnourished patients available for the present study were considered to be infected on admission. Those who were infected were placed on broad spectrum antibiotic therapy which included one or more of the following antibiotics depending on the site of infection: ampicillin, methicillin, gentamycin and cephalothin.

The patient's 71-day hospital course was divided into 3 main periods. During the stabilization period (days 1-7), all patients were given a gradually increasing caloric intake, from 25 calories-1 g protein per kilogram per day at day 1 to 100 calories-1 g protein per kilogram per day at day 7. The stabilization period was followed by a 3-week study period (days 8-29) when the patients were randomly assigned to 1 of 4 dietary treatment schemes (Table 1). All patients again received the same diet (175 calories-4 g protein per kilogram per day) after hospital day 29. In addition to the above milk-base formula diets, a complete vitamin and mineral supplement was given starting on day 2.

*Collections of specimens:* Blood was collected on days 1, 8, 29, and 71 by venipuncture and allowed to clot at room temperature. Serum was collected and immediately frozen at -20°C for a period of up to 3 months before analysis. In some cases, only plasma was available. Because both serum and plasma give identical results in the analysis for the complement and the C3PA levels by radial immunodiffusion, for ease of communication, they are referred to as serum throughout the remainder of this report.

*Quantitative determination of individual complement proteins and C3-proactivator:* The concentrations of C1q, C1s, C3, C4, C5, C6, C8, C9, and C3PA were measured by the radial immunodiffusion technique using immunoplates containing antiserum specific for individual complement components and for C3PA. The immunoplates and the reference standards were kindly supplied by Dr. H. J. Muller-Eberhard (Scripps Clinic and Research Foundation, La Jolla, Calif., U.S.A.). Seven microliters of reference standards and test specimens, appropriately diluted in a pH 8.0 isotonic buffer that contained 0.1M sodium chloride, 0.03M potassium phosphate, and 0.01 M trisodium ethylenediaminetetraacetate, were placed in the antigen wells. The plates were incubated at room temperature for 48-72 hours, and the precipitin rings were measured directly with a calibrated magnifier instead of from photographs as originally described. The reproducibility of the technique, as performed in this laboratory, was between 15 and 20%. All specimens were provided in coded form. The values reported for each specimen were the average of values determined at 2 different serum dilutions.

## RESULTS:

*Complement and C3-proactivator levels in malnourished children:* As shown in Figure 1, the mean concentrations of 7 of the 8 complement proteins (C4 excepted) and of C3PA in the serum obtained on day 1 from 10 marasmic and 10 kwashiorkor children were significantly lower than the means of samples from 19 normal children of similar age residing in the same area (p less than 0.05). The mean concentration of C4 was less than that of controls, but the difference was not statistically significant (p greater than 0.05). The 8 complement proteins did not appreciably increase during the initial 8 day stabilization period. However, on day 29, a marked increase in the level of all components except C9 was observed. The levels of C1q, C1s, C3, C4 and C5 reached maxima on days 29 or 71 which were significantly higher than the normal mean. It appeared that the relative rise in concentrations of the late-acting components, ie., C5, C6, C8 and C9, was not as dramatic as that of the early-acting components, particularly C3 and C1s. The level of C9 did not return to normal during the 71-day period. The concentration of C3PA reached normal levels by day 29. The composite results shown in Figure 1 were next analyzed according to clinical groups and to the types of dietary treatment.

*Comparison of complement and C3 proactivator levels in marasmic and kwashiorkor children:* The admission levels of 7 of the 8 complement proteins (C4 excepted) and of C3PA in the 10 children with kwashiorkor were lower than the corresponding levels of the 10 marasmic children of the same age (Fig. 2). The differences between the 2 groups were statistically significant at the 5% level only for C1q, C6, and C8. Of the 9 proteins analyzed, only the concentrations of C5, C6 and C3PA from the marasmic group were

significantly lower than the "normal" controls ( $p$  less than 0.05), suggesting that malnourished children of the marasmic type were less severely affected by PCM. However, by day 8 the complement and the C3PA levels were similar in both clinical groups and remained so throughout the study.

*Effects of dietary treatment on the recovery of complement and of C3 proactivator levels:* Because preliminary analysis of the data discussed above indicated that the children with marasmus and kwashiorkor responded similarly to dietary treatment, the data from these 2 groups were combined for analysis of the effects of the different dietary treatments. The 20 children were divided into 4 dietary groups as shown in Table 1.

The quantity of dietary protein markedly influenced the levels of complement proteins and C3PA (Fig. 3). The concentrations of the complement proteins and C3PA of the high and low protein dietary groups were both low before institution of special dietary treatment on day 8. The stimulating effect of higher protein intake was apparent on day 29, 3 weeks after the start of the treatment diets; the children who received 4 g of protein per kilogram per day (group 4) had reached normal or above normal complement levels, whereas the children who received 1 g of protein per kilogram per day (group 3) had not only failed to increase their complement levels, but also suffered a further reduction of some components. Forty-one days after their diet was changed from 1 g to 4 g of protein per kilogram per day, the low protein group showed significant improvement in the complement levels (day 71). As shown in Figure 3, the concentrations of the 7 complement proteins (C4 excepted) and of C3PA of both dietary groups were indistinguishable on day 71.

The effect of caloric intake on the recovery of the complement and C3PA levels was not as dramatic as that for protein (Fig. 4). Although the number of cases was small, the children who received the high caloric diet (group 4) tended to respond better than those on the low caloric diet (group 2).

Because only 2 children comprised diet group 1, the changes after dietary treatment may not be representative of the group. It appeared, however, that some improvement of the complement levels was achieved on a diet of 100 calories—1 g protein per kilogram per day. The recovery seemed to be slower and less complete than in the other groups.

*Effect of infection on the complement profile in PCM children:* Serum samples taken from 2 infected, malnourished children a few days before, or on the day of death showed the complement and the C3PA levels to be markedly depressed (Table 2). All complement components and C3PA were noticeably lower than those from PCM children who recovered. Many components, in fact, dropped below the lower limits of sensitivity of the detection method.

#### SUMMARY AND CONCLUSIONS:

The serum levels of complement proteins (C1q, C1s, C3, C4, C5, C6; C8, and C9) and of C3 proactivator in 20 children with protein-calorie malnutrition were compared with those of "normal" children of the same age residing in the same geographical area. These components were determined on admission and at intervals during different dietary treatment regimens. All components, except C4, were markedly lower in the malnourished children on admission than in the "normal" children, and the children with kwashiorkor were more severely affected than the children with marasmus. The quantity of dietary protein, and to a lesser extent the caloric intake, had a marked influence on the repair of the complement system.

Table 1. Dietary treatment and clinical status of PCM children.

Dietary Group	Dietary Intake		No. Children		
	Calories/kg/day	G protein/kg/day	Marasmus	Kwashiorkor	Total
1	100	1	1	1	2
2	100	4	3	1	4
3	175	1	3	3	6
4	175	4	3	5	8

Table 2. Complement and C3PA levels in malnourished children who died during hospitalization.

Patient	Cause of death	Hospital Day	Per cent of normal							
			C1q	C1s	C3	C5	C6	C8	C9	C3PA
A	Sepsis	1	<18	30	12	40	32	<36	37	46
		3 (Immediately before death)	<18	30	10	37	28	<36	22	28
B	Sepsis	2 (One day before death)	<18	<18	5	<16	14	<36	<11	<13
PCM children (Mean values from 20 subjects who recovered)		1	84	69	63	79	59	75	55	61

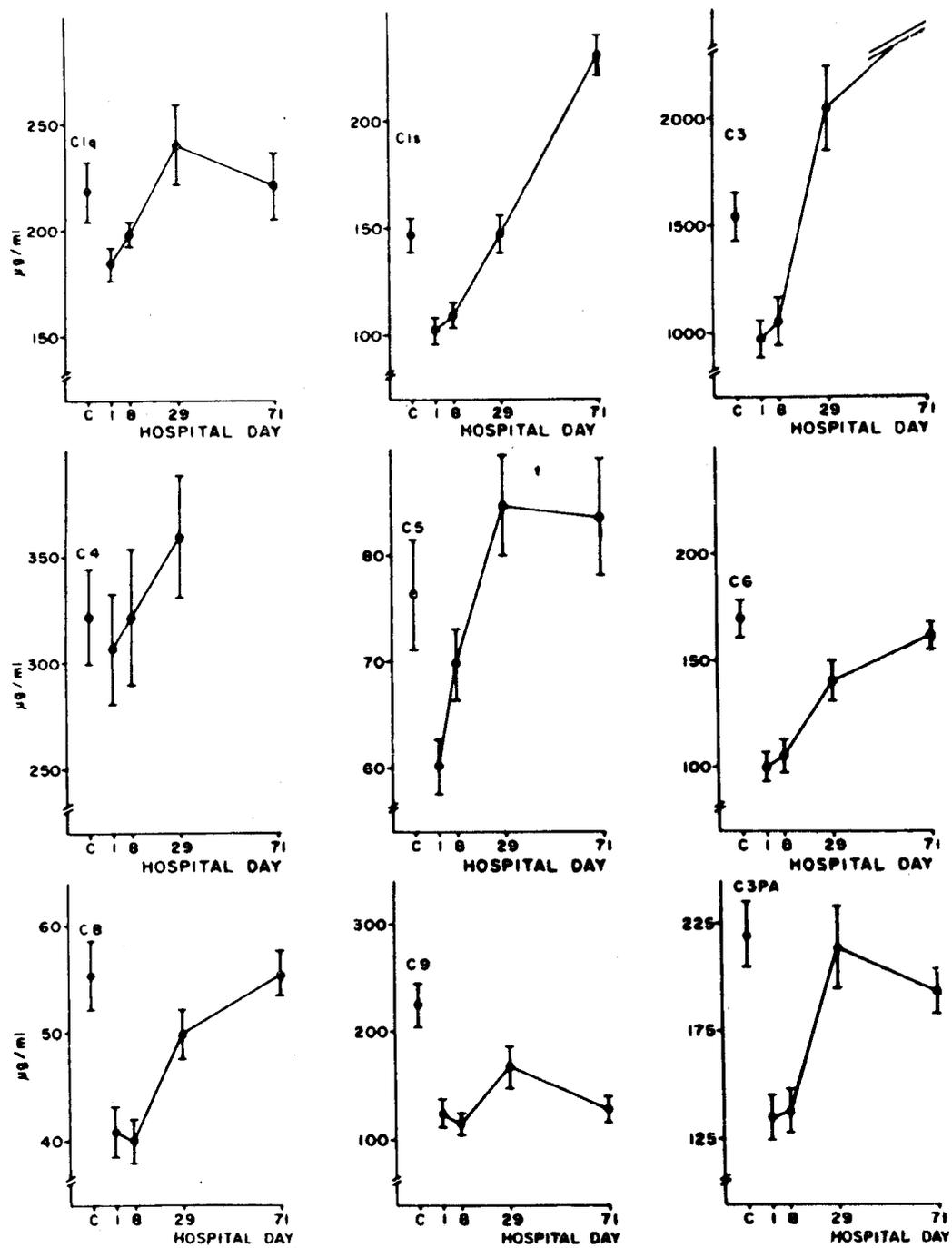


Figure 1. Mean Concentrations of Complement Proteins in Children with Protein Calorie Malnutrition.

ADMISSION SERUM COMPLEMENT LEVELS IN MARASMUS AND KWASHIORKOR CHILDREN

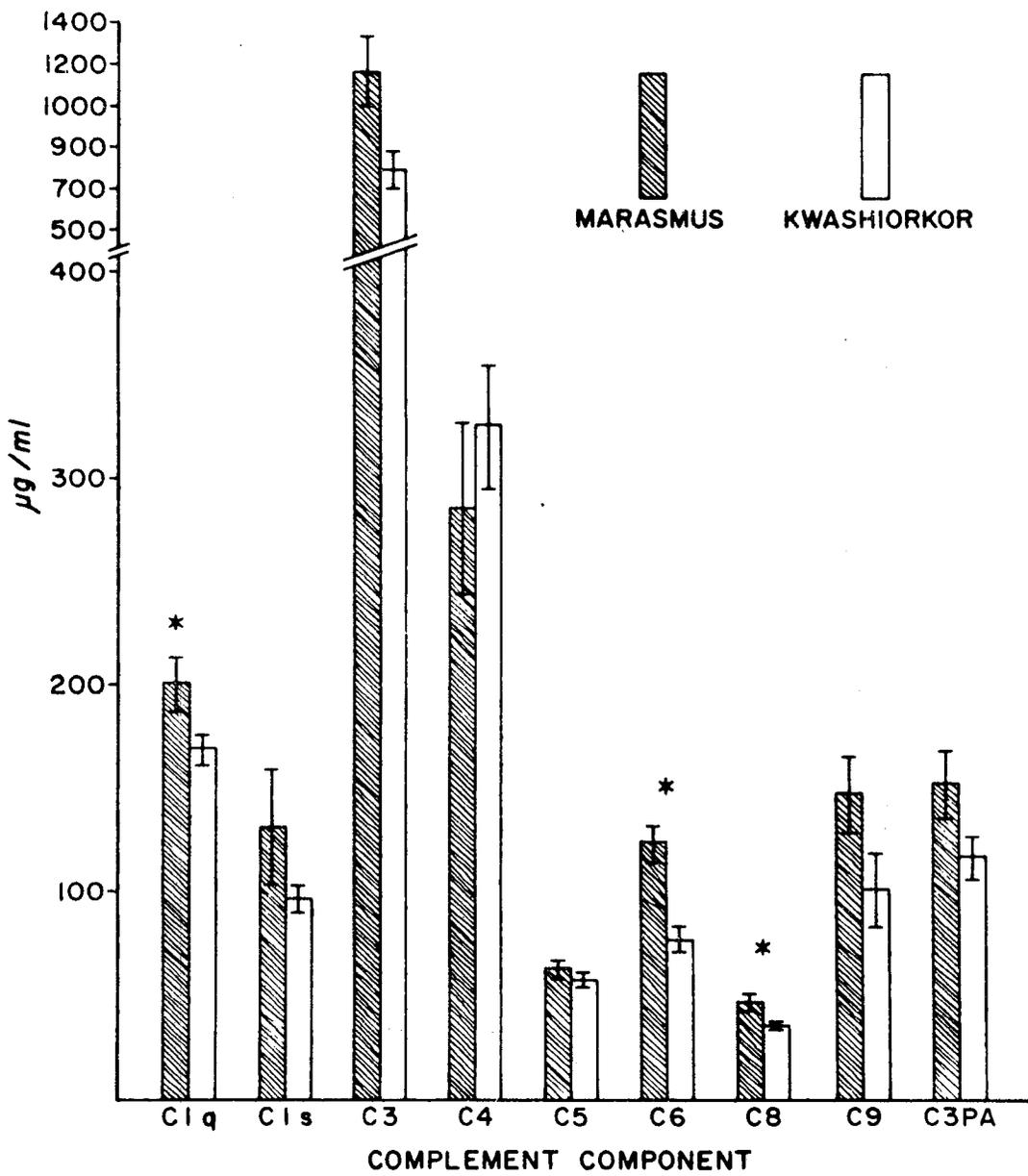


Figure 2. Mean Admission Concentrations of Complement Proteins In 10 Children with Kwashiorkor and Marasmus.

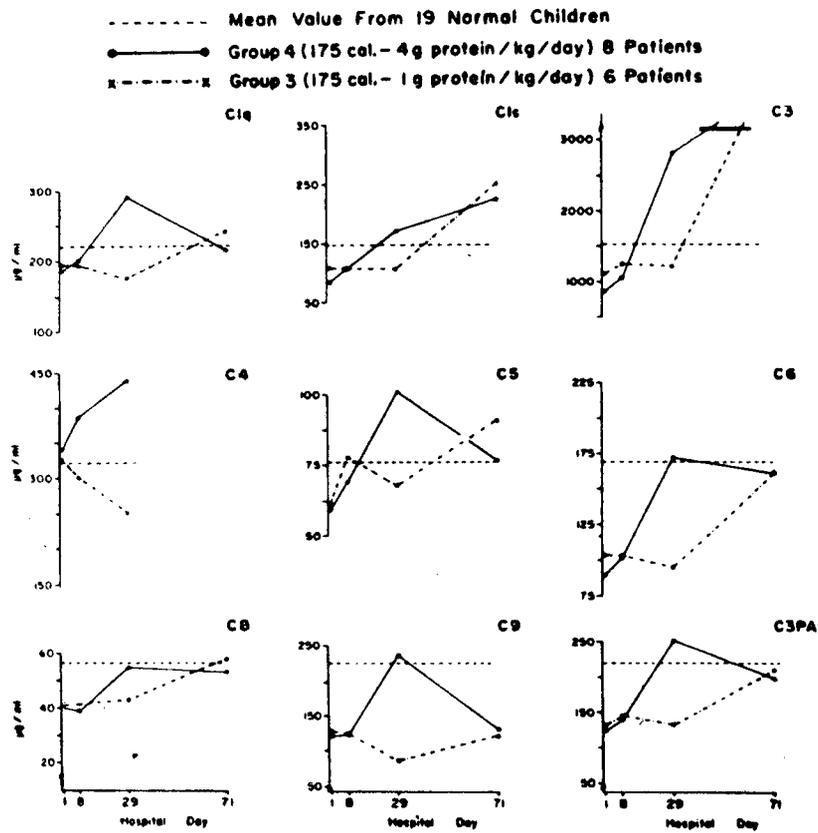


Figure 3. Effect of Amount of Dietary Protein on Serum Complement Protein Concentration.

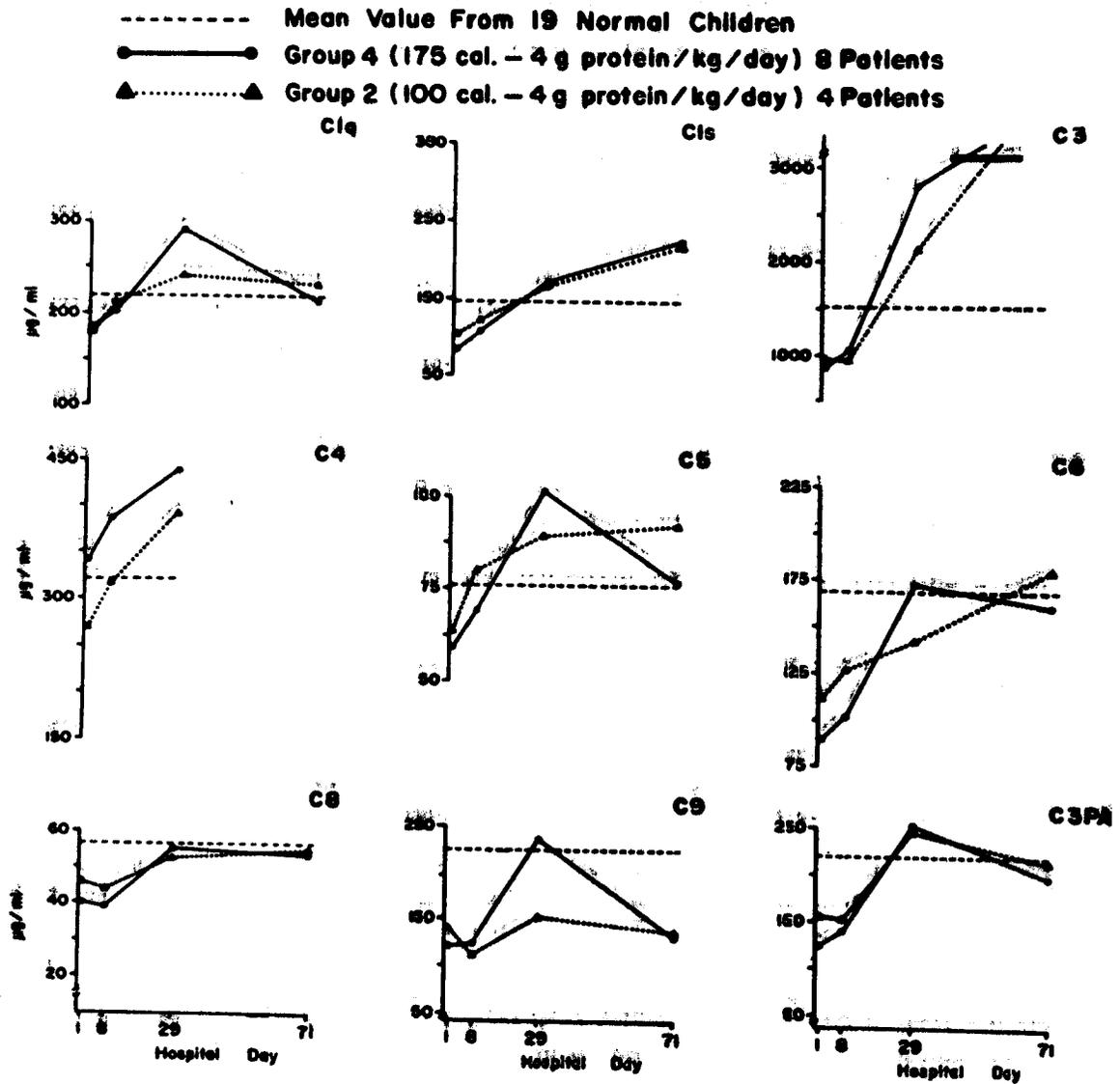


Figure 4. Effect of Caloric Intake on Recovery of Complement Protein Levels.