DISCUSSION
The present work has been carried to study the complement activity in 22 pre-school children (1-5 years age) suffering from protein-calorie malnutrition and 12 nutritionally normal age matched children, serving as control. The study was conducted at M.L.B. Medical College, Jhansi between May 1981 and March 1982.

The primary aim of our study was to evaluate the complement profile in children suffering from PCM and compare the values with those obtained in control cases. Besides evaluating the complement activity traditional parameters viz. weight, length/height and mid-arm circumference, serum albumin and blood haemoglobin were used to assess the nutritional status of children. Complement activity was assessed by total haemolytic complement (CH50) activity, alternative pathway activity (AP50) and C3 concentration. It was also our endeavour to ascertain the possible inter-relationship between the clinical progress, following nutritional rehabilitation and the subsequent change in complement activity. With the objective in view, complement activity, anthropometric measurements, serum albumin and blood haemoglobin values were evaluated at the time of initial contact and in subsequent follow ups at 2 weeks, 4-7 weeks and 10-12 weeks interval. Statistical analysis was done to derive means and standard deviations (SD). An attempt was made to correlate age, weight, length/height,
mid-arm circumference, serum albumin and blood haemoglobin with various parameters of the complement activity.

The PCM group in our study was further classified into 19 cases of marasmus, 13 cases of marasmus-kuashorkor and 3 cases of kuashorkor according to McLean classification. All the cases belonged to low socio-economic status and most of them were suffering from gastrointestinal and respiratory tract infections. Care was however taken to exclude the cases in whom secondary factors thought to affect complement activity could have been operational.

History of past illness and family history were noted in each case. All the children at the initial contact were receiving diet, grossly deficient in calories and proteins. None of the twelve control cases was suffering from any demonstrable illness at the time of inclusion in this study.

Based on observations depicted in tables I to XVII, various inferences have been drawn and discussed under different headings.

**ANTHROPOMETRIC PROFILE**

Anthropometric profile of both the control and PCM cases at the time of initial contact (Table III) revealed that the mean weight, length (height) and mid-arm circumference in all 3 groups of PCM viz. marasmus, marasmus-kuashorkor and kuashorkor were appreciably less than those in healthy controls. However, groups of PCM among themselves did not show clear differences in anthropometric values. Subsequently,
during follow up, after nutritional rehabilitation and subsidence of infection, it was seen that mean weight and mid-arm circumference increased consistently during 1st, 2nd and 3rd follow ups in cases of marasmus and marasmic-kwashiorkor and during the 1st follow up in a single case of kwashiorkor. It was also observed that the sustained improvement in weight and mid-arm circumference was more marked in cases of marasmus. However, there was no improvement seen in mean length (height) at subsequent follow ups in the 3 groups of PCM cases, exception being a single case of kwashiorkor. These findings are not surprising since it is known that body weight is a more sensitive indicator of nutrition and that evaluation of linear body change is usually possible at longer intervals of time.

Correlation of Weight (expressed as % of 50th percentile of Harvard Standard) with Complement Activity:

From Table XIII it is obvious that no definite trend of rise or decline was seen in CH₅₀ values in relation to change in percentage weight. Correlation coefficients of percentage weight with CH₅₀ and AP₅₀ were 0.192 and 0.099 respectively, being non-significant. However, a positive significant correlation (r = 0.685) was seen between the percentage weight and CS values.

Correlation of Length/Height (expressed as % of 50th percentile of Harvard Standard) with Complement Activity:

Correlation coefficient of length (height) percentage
with both CH50 and AP50 values was non-significant, while C3 values \( r = 0.636 \) was statistically significant, as shown in Table XIV.

Correlation of Mid-arm Circumference with Complement Activity:

The correlation of the mid-arm circumference with complement activity (Table XV) revealed that there was no definite rise or decline in CH50 and AP50 values with the change in mid-arm circumference, correlation coefficients \( r \) being 0.365 and 0.143 respectively. As with weight and length/height, it was observed that there was a definite rising trend in C3 values in relation to arm circumference, correlation coefficient being 0.721.

Thus we see that the percentage of weight, height (length) and mid-arm circumference had a positive and significant correlation with complement C3 levels, while CH50 and AP50 values had no significant correlation with anthropometric measurements. Our observations are comparable with the findings of other workers in the field. Olani et al (1976) in their study to evaluate the complement profile in PEM cases, noted that with refeeding, C3 was the first complement component to show a significant rise, followed by C9 and C6, thereby concluding that out of all the complement components, C3 was the most sensitive index of nutritional status, Kielmann et al (1976), in their study of complement profile in pre-school children has also shown a positive correlation of weight, length (height) and arm circumference with C3 values. Thus the findings of the present study and that of other workers clearly reveal that weight and length (height)
percentage, and arm circumference show a positive correlation with only one parameter of complement activity viz. C3 levels, suggesting that with nutritional rehabilitation, there is not only an improvement in the growth and development, but also an improvement in the immunological status of the child.

**Serum albumin and haemoglobin values:**

It is obvious from Table IV that the mean serum albumin and haemoglobin values were significantly depressed in PCM cases as compared to the controls ($P < 0.001$). Similar findings have been observed by other workers in the field (Olusi et al, 1976 and Haller et al, 1978). Further it was observed that mean serum albumin and haemoglobin values were lowest in cases suffering from kwashiorkor (Table V and Fig. 5) at the initial contact. After nutritional repair there was a sustained rise in mean serum albumin levels, during the 3 follow ups, marasmus, marasmic-kwashiorkor cases. Similarly rise in serum albumin was seen during the 1st follow up in a single case of kwashiorkor (Table IX, X and XI). Like serum albumin level, mean haemoglobin level also increased substantially in the all 3 follow ups in marasmus and marasmic-kwashiorkor and during the 1st follow up in single case of kwashiorkor. Thus it was apparent that nutritional repair, with adequate amount of calories and proteins, led to a sustained rise of serum albumin and haemoglobin values in all groups of PCM. Evaluation of these two parameters of nutrition and their
correlation with the complement activity has not hitherto been attempted by other workers and hence comparison to other studies could not be done.

**COMPLEMENT PROFUSE IN STUDY GROUPS:**

**I CONTROLS:**

Total Haemolytic Complement (CH<sub>50</sub>) activity:

In the present study, total haemolytic complement (CH<sub>50</sub>) had mean values of 7.16 ± 1.93 U/ml. It was seen that values from our study were much lower than those obtained by other workers. Smythe et al. (1971) found CH<sub>50</sub> values in the range of 1/128 - 1/512. Chandra (1973) and Jagadeesan and Reddy (1979) reported mean values of 38.00 ± 13.00 and 66.10 ± 8.31 U/ml respectively. However, Sushini et al. (1976) found very high values of CH<sub>50</sub> viz. 340.00 ± 50.00 U/ml in their study. The possible explanation for wide variation in CH<sub>50</sub> values, obtained by various authors, could be that estimation of CH<sub>50</sub> depends upon the standardization techniques used in different laboratories.

Since all control cases in our study were free of infections, we could not predict the effect of infection on the value of CH<sub>50</sub> in well-nourished children.

**Alternative Pathway Activity (AP<sub>50</sub>):**

In our study mean value of AP<sub>50</sub> were found to be 64.70 ± 10.11 U/ml. Inspite of our best efforts reference values of AP<sub>50</sub> (a measure of alternative pathway activity) could not be found in literature for comparative study.
Complement C3 Values:

C3 complement values (mg/dl), considered to be most sensitive index of complement activity, had mean values of 135.83 ± 23.98 in normal control children. These values were found to be consistent with those obtained by various other workers viz. Chandra (1972), Siriwitsa et al (1973), Chandra (1975), Olasi et al (1976), Kielmann et al (1976), Wellor et al (1978) and Jagadeesan and Reddy (1979). However Newman et al (1975) found slightly lower values of C3 i.e. 96.90 ± 2.40 mg/dl.

Chandra (1975), in his study to evaluate the serum complement levels in malnutrition and well-nourished children, observed that the complement C3 value was much higher (345.00 ± 57.00 mg/dl) in well-nourished children with infection than the other group of well-nourished children without infection (132.00 ± 16.00 mg/dl). The author however could not ascribe any explanation of the heightened C3 levels with infection in well-nourished children. Since all the 12 control children in our study were free of infection, we could not arrive at a definite conclusion regarding the effects of infections on C3 complement levels.

II PROTEIN-CAJORIC MALNUTRITION:

INITIAL CONTACT:

Total Haemolytic Complement (CH₅₀) Activity:

It is evident from Table IV that at the time of initial contact, mean CH₅₀ value in the PCN group was lower
than the value in control group, the difference between the
two values was statistically non-significant ($P < 0.05$).

On further analysis it was observed that though the
values of CH$_{50}$ in marasmus, marasmic-kuashierker and
kuashierker were lower than the controls (Table V and Fig. 6),
these values were not significantly different from the controls
($P > 0.05$). Similarly the group differences of mean CH$_{50}$
values were not appreciably different from each other. In
contrast to our study, Smythe et al (1971) and Chandra (1975)
reported significantly lowered values of CH$_{50}$ in infants and
children with PGM. Suskind et al (1976), in an elaborate
study to assess the complement activity in 28 children with
severe PGM, using the CH$_{50}$ titre as the parameter, reported a
significant depression of CH$_{50}$ levels, only in cases of
kuashierker, as compared to the controls. However, on
comparing the CH$_{50}$ values of children with marasmus and
marasmic-kuashierker to those of controls, they did not
observe any significant difference. Waller et al (1978) and
Jagadeesan and Reddy (1979) have also observed a more marked
depression of CH$_{50}$ values in kuashierker than in marasmus
and marasmic-kuashierker cases.

The results of the present study therefore reveal,
that, though CH$_{50}$ values were lower in all groups of PGM, a
statistically significant difference as compared to controls,
could not be elucidated. Further, unlike other workers, we
could not arrive at any correlation between the CH$_{50}$ values
and the severity of PGM; values in all 3 groups remained
were or less equal. This was in contrast to the results obtained by other workers, where not only \( \text{CM}_{50} \) values in PCM were statistically different from the controls but maximum depression was also observed in the most severe form of PCM viz. kushielker.

Alternative Pathway Activity (\( \text{AP}_{50} \)):

It is evident from Table IV that the mean \( \text{AP}_{50} \) values in the PCM group were not statistically different from the controls. Further, the mean \( \text{AP}_{50} \) values in warasawa, waramko-kushielker and kushielker were found to be 63.83 \( \pm \) 24.91, 63.38 \( \pm \) 23.83 and 51.40 \( \pm \) 3.90 respectively (Fig. 7). Although these values in 3 groups of PCM were lower, only the value in kushielker group was significantly lower (\( p \leq 0.05 \)) as compared to the controls. This suggests that the alternative pathway activity was adversely affected only in the kushielker group, values being unaffected in warasawa and waramko-kushielker cases. Since there is paucity of data regarding \( \text{AP}_{50} \) in literature, a comparison of these values could not be ascertained.

However, Sirisinha et al (1973) and Maller et al (1979) studied the alternative pathway activity by evaluating the concentration of factor B (named C3 pro-activator, previously) and observed that this factor was depressed in children suffering from PCM. Since these authors found normal C4 levels, which are depressed in classical pathway activity, they suggested that in PCM, alternative pathway was also activated. However, in the present study, alternative pathway activity was found to be unaffected except in kushielker. The possible mechanism of depressed \( \text{AP}_{50} \) (alternative pathway activity) values could be that
infection triggered alternative pathway thereby resulting in diminished concentration of \( \text{AP}_{50} \) values. Since in our study, all the cases of kwashiorkor were severely infected, maximum depression of \( \text{AP}_{50} \) values was obtained in this group. Further, a significant reduction in alternative pathway activity could have accounted for lowering the antimicrobial resistance therefore resulting in severe infection in kwashiorkor.

**Complement C3 Values:**

The complement C3 levels in FCM group had mean value of \( 60.39 \pm 23.25 \) mg/dl. These values were found to be statistically lower than those in controls (Table IV). On further analysis of the C3 levels in marasmus, marasmic-kwashiorkor and kwashiorkor it was observed that the values in all 3 groups were significantly lower as compared to controls (\( P < 0.001 \)), maximum depression being observed in cases of kwashiorkor. However, the mean values in the 3 groups of FCM were not appreciably different from each other (Table V and Fig. 8). A significant depression of C3 level in FCM cases was also obtained by various other workers in the field viz. Chandra (1973), Sirisinha et al (1973), Chandra (1975), Neuman et al (1973), Okusi et al (1976), Kielmann et al (1976), Haller et al (1978), Kielmann and Curcio (1979) and Jagadesean and Reddy (1979).

It was further observed in the present study that a definite correlation existed between the serum albumin concentration and complement C3 levels (\( r = 0.895 \)). In the
kwashiorkor group concentration of serum albumin was the lowest and maximum depression of the C3 level was also obtained in these cases (Table V), thus suggesting a direct correlation of serum C3 values with the severity of malnutrition.

**Correlation of Age with Complement Activity**

No definite trend of rise or decline was seen in complement values viz. CH$_{50}$, AP$_{50}$ and C3 in relation to age, thus suggesting no effect of age on complement activity. This aspect has not been evaluated so far by any other worker.

**Correlation of Serum Albumin Values with Complement Activity**

It is evident from Table XVI, that when serum albumin levels were correlated with the CH$_{50}$, AP$_{50}$ and C3 levels, a definite and positive rise was observed only in CH$_{50}$ and C3 levels with the increasing levels of albumin. Correlation coefficients ($r$) of serum albumin with CH$_{50}$ and C3 were 0.399 and 0.625 being significant at $P \leq 0.05$ and $P < 0.001$ respectively. However, no rise in AP$_{50}$ values was observed with the increasing levels of serum albumin ($r = 0.107$).

On further evaluation, it was observed that there was a positive correlation ($r = 0.346$) of C3 with CH$_{50}$ values, being statistically significant ($P \leq 0.05$). However, on comparison of C3 and CH$_{50}$ values with AP$_{50}$ values, correlation coefficients ($r$) were found to be -0.029 and -0.223 respectively, both being non-significant ($> 0.05$).
A positive significant correlation between C3 and CH₅₀ values implies that a change in the concentration of C3 will reflect on CH₅₀ values. In this regard, Spitzen (1977b) reported that CH₅₀ values were affected only when there was approximately 50% decrease in C3 levels. However, since negative non-significant correlation was observed between C3 and AP₅₀ values, the values of these two parameters would otherwise be independent of each in PGN cases.

A similar significant correlation between serum albumin levels and complement activity was also observed by Sirisinha et al. (1973), Haller et al. (1978) and Jagadeesan and Reddy (1979). They reported that there was a direct correlation between the degree of complement depletion (especially C3 levels) to the severity of the depletion of various plasma proteins.

Sirisinha et al. (1973), Haller et al. (1978) and Jagadeesan and Reddy (1979) thus suggested that decreased protein synthesis in the liver played a major role in the reduction of C3 levels and hence in the impairment of complement system in PGN. However, Kielmann and Carnie (1979) showed that there was no significant correlation between total serum proteins and C3 levels.

Correlation of Hemoglobin Values with Complement Activity:

An attempt was made to correlate the hemoglobin values with the complement activity (Table XVII). It was observed that there was no definite rising or declining trend
in CM50 and AP50 values in relation to haemoglobin values, correlation coefficients (r) being 0.114 and 0.196 respectively (P > 0.05). However, a significant positive correlation (r = 0.628) was observed between haemoglobin and C3 values.

A significant correlation between haemoglobin and C3 values in our study suggest that haemoglobin per se, could also be one of the factors responsible for depression of C3 levels in PGM cases. However, a study conducted by Kielmann and Cureio (1979) did not show any significant correlation of C3 levels with haemoglobin values.

**Complement Activity and Infection:**

In the present study since all the PGM cases were suffering from infection, it was not possible to elucidate whether there was any subsequent difference in the complement activity between infected and non-infected cases. Sirisinha et al (1973), Chandra (1975), Sashind et al (1976) and Kielmann and Cureio (1979) have all reported that there was pronounced depression of complement activity (especially CM50 and C3 levels) in PGM children suffering from infection than in those without it. Further they also reported difference in the complement levels with severity of infection, levels being lower in severely infected cases than in those having mild infection.
FOLLOW UP:

After the initial assessment of anthropometry, serum albumin, haemoglobin and complement activity in the FCM cases, all of them were put on nutritional rehabilitation schedule and infections were treated by appropriate antibiotics. An attempt was made to follow all FCM cases with repeat anthropometry, estimation of serum albumin, haemoglobin and complement activity.

Total Haemolytic Complement (CH50) Activity:

It is evident from Tables IX, X and XI that after nutritional repair, CH50 level attained maximum mean values after the 1st follow up in all the cases of marasmus and marasmus-kwashiorkor cases and also in a single case of kwashiorkor. Although in the 2nd and 3rd follow up, mean levels had a declining trend in both marasmus and marasmus-kwashiorkor cases, yet the levels remained higher than that obtained at initial contact. Since the solitary case of kwashiorkor left the hospital after 1st follow up, subsequent CH50 level could not be ascertained. Our results are consistent with the findings of Chandra (1973), Sushied et al (1976) and Jagadeesan and Reddy (1979).

Alternative Pathway Activity (AP50):

Tables IX, X and XI clearly demonstrate that on evaluation of AP50 values at follow up in different groups of FCM, no consistent pattern of rise was observed. Since there is a paucity of available literature on AP50 values, comparison of our values could not be elucidated.
Complement C3 Values:

It is evident from Table IX, X and XI, and Fig. 9, 10 and 11 that after nutritional rehabilitation C3 values, attained maximum mean level after the 1st follow up, in only marasmic-kwashiorkor. Values in marasmus showed a definite increasing trend till the 2nd follow up. In a single case of kwashiorkor followed only once, significant rise of C3 level was observed. Rise of C3 levels after nutritional rehabilitation was also observed by Sirisinha et al (1973), Chandra (1975), Olusl et al (1976) and Jagadeesan and Reddy (1979).

A critical analysis of the complement activity on follow up in the 3 groups of FCM revealed that the levels of most complement components, after nutritional rehabilitation, rose to above control values. The mechanism involved in such overshoot or rebound is still not known. However, Sirisinha et al (1973) are of the view that after complement depletion in FCM, there is an accentuated synthesis of the complement proteins and a state of over production ensues, accounting for above the normal values of complement components.

Thus the findings on the complement activity in children suffering from protein-calorie malnutrition, in the present study, reveal certain interesting observations. That the complement profile was significantly depressed in FCM cases as compared to the controls. It was seen that the
Depression of complement activity was mainly accounted by a significant depression of the complement C3 level, while CH50 and AP50 values, though lower were not statistically significant from the controls. Another significant finding of our study was highly significant depression of complement C3 ($V \leq 0.001$) in all 3 groups of BCM, levels being maximally depressed in kwashiorkor. However, though CH50 values were lower in all the groups, no statistical significance was observed as compared to the controls ($V \geq 70.05$). AP50 values, on the other hand, were found to be depressed only in kwashiorkor ($V \leq 0.05$), while no significant difference was obtained in marasmus and marasmus-kwashiorkor. Since C3 levels were found to be depressed in all forms of malnutrition, it was inferred that probably complement C3 was the most sensitive index of nutritional status. The findings, that CH50 levels were more or less equal in all the groups of BCM and also did not show any significant difference from the controls, demonstrated that this parameter was not a very good index of evaluation of complement activity, a change in CH50 levels could possibly be affected by at least a 50% reduction in C3 levels (Spitser, 1977b).

In the present series an attempt was made to evaluate the various factors which could possibly have an adverse influence on the complement profile. On the basis of a positive correlation of nutritional status (measured by
anthropometric indices), serum albumin and haemoglobin values with the complement activity, we arrived at the following possible explanations for the depression of complement system:

1. **Reduction in Protein Synthesis**

   This was the single most important factor as observed in present study to cause depression of complement activity. This finding is substantiated by the following observations:

   (a) There was a uniform depression of serum albumin levels in all the groups of PCM. Further it was observed that greater the reduction of albumin levels, more significant was the depression of complement activity, the correlation coefficient between serum albumin and C3 levels being highly significant (r ≤ 0.001).

   (b) With nutritional repair, there was not only a substantial increase in serum albumin levels but also concomitant rise in complement activity.

   These observations in our study suggested that nutritional status of the individual could influence the complement system.

2. **Reduction in Haemoglobin Values**

   Since a definite correlation was observed between haemoglobin levels and complement C3 activity, one could suggest that haemoglobin levels played an important role in the depression of complement levels.
Various hypothesis have been put forward by different workers to explain the possible mechanisms of depression of complement activity in PCM. Sirisinha et al (1973), Chandra (1975) and Sushkind et al (1976) are unanimous in their opinion that the child's nutritional status was probably the major factor in causing the depression of complement activity. Chandra (1975) further stated that since liver damage occurred in PCM and liver was the main site of C3 synthesis, reduction of C3 levels could be a corollary to liver damage. Another important factor to cause depression of complement activity could be a phenomenon of complement consumption which occurred in the presence of infection. Sushkind et al (1976) suggested that anticomplementary activity in serum of PCM cases could also contribute to depression of CH50. Other possible explanations for depression of complement system in malnourished children, though unimportant, could be the changes in blood vascular compartment occurring in malnutrition (Chandra, 1975). Complement depletion could also occur as a result of protein-losing gastroenteropathy in PCM cases (Chandra, 1975).

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