REVIEW OF LITERATURE
PROTEIN-CALORIE MALNUTRITION

Historically, marasmus (Greek marasmos, wasting) was recognised for hundreds of years as being, with gastroenteritis, a major contributor to high infant mortality. In the early part of this century reports from central Europe of so-called 'Starch dystrophy' attracted little attention. The classic description by Williams (1933) of a disease attributable to protein deficiency, which he named 'kwashiorkor' (taken from the 'Ga' language of Ghana), recognised that this was the disease, the first child got when the second was on the way. It was characterised by skin and hair changes, oedema, moonface, fatty liver, hypalbuminaemia and psychomotor changes. Clinical descriptions of a disease, obviously similar, appeared from many other countries although in the West Indies for example, the dermatosis was uncommon, oedema prominent and the term 'Sugar baby' was subsequently used by Waterlow (1948) and Jelliffe et al (1954).

Jelliffe (1959) coined the term 'Protein-Calorie Malnutrition (PCM)' of early childhood to include the mild and moderate degrees and all the clinical types of the severe degree of malnutrition. Using a variety of biochemical tests McLaren et al (1967) were able to show that the severe degree of PCM in its various clinical forms of marasmus, marasmic-kwashiorkor and kwashiorkor formed a spectrum of both clinical signs and biochemical changes; both being most marked in full blown kwashiorkor and least evident in pure marasmus.
There was a short lived effort through World Health Organisation (WHO) to introduce the term 'Protein-calorie deficiency diseases' but this was abandoned by the expert group meeting in 1970 in favour of PCM. Since then, the impact of proposals to replace the term calorie by joule as a unit of energy measurement has led to a general use of the word 'Protein-Energy Malnutrition' rather than Protein-Calorie Malnutrition. At present there is an increasing recognition of the fact that the major problem all over the world is deficiency of food intake in general (and therefore of energy) rather than of protein in particular. Again, to emphasize that this is but part of the overall energy crisis of mankind, the term energy-protein malnutrition or EPM has been used by workers to give the needed stress to energy deficit (McLaren, 1976).

Magnitude of the Problem:

PCM is one of the world's major public health problem especially in the underdeveloped and developing countries. Rao et al (1969), in a study of pre-school children of rural communities, found that the percentage prevalence of frank cases of kwashiorkor and marasmus were 0.6 and 1.0 respectively. In a survey of rural pre-school children Ghai et al (1970) reported that about 18 percent of all cases were undernourished. Out of these 1.7 percent had nutritional marasmus and 0.9 percent suffered from kwashiorkor.
Point prevalence figures have been collected for a number of years by WHO. Bongoa (1974) reported data from 77 nutrition surveys in 46 developing countries, totalling near 2 lakhs children mostly under 5 years of age and suggested that about 100 million children throughout the world were suffering from moderate or severe PCM at any one time. Distrian et al (1974) have brought attention to the large number of patients with secondary malnutrition who were present in the wards of United States hospitals.

Gopalan (1974) computed that nearly 65 percent of toddlers in poor communities in India suffered from moderate malnutrition and 18 percent from severe malnutrition. Ghai (1975) analysed severe cases of PCM in hospitals and reported 6.6 percent deaths in cases suffering from marasmus and 33.3 percent in kwashiorkor and marasmic-kwashiorkor.

Ghai (1977) showed that malnutrition was a major contributory cause of mortality in about 40 percent of childhood deaths, even though it was often not listed as a primary cause of death in most of studies. Rao (1978) reported that marasmus and kwashiorkor were seen only in 1-3 percent of the pre-school child population. As many as 60-70 percent of the children, on the other hand, suffered from mild and moderate degree of PCM.
Recently Ghosh (1981) has shown that in India, there were about 100 million pre-school children out of which 3 to 4 million suffered from severe types of malnutrition, and probably 1 million of them died.

**Classification (Grading):**

Grading of PCM is necessary for formulating therapy in individual patients and defining priorities for combating malnutrition. Three main direct methods of assessing PCM in the community have been used: clinical, anthropometry and biochemical.

Gomes et al. (1955) is credited with the first ever classification of malnutrition, using the actual weight expressed as a percentage of standard weight (Boston 50th percentile) for age. The presence or absence of clinical characteristics such as oedema was not taken into account by the authors.

<table>
<thead>
<tr>
<th>Grade of malnutrition</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&gt; 90% of expected weight for age.</td>
</tr>
<tr>
<td>Mild (1st degree)</td>
<td>89 - 75%</td>
</tr>
<tr>
<td>Moderate (2nd degree)</td>
<td>74 - 60%</td>
</tr>
<tr>
<td>Severe (3rd degree)</td>
<td>&lt; 60%</td>
</tr>
</tbody>
</table>

In a later modification, Jelliffe (1966) included all cases with nutritional oedema, irrespective of weight, in 3rd degree.
Although weighing scales may not always be available or maintained or used correctly and age is often not known accurately, yet this method is in common use. Its main drawbacks are, that it assumes all children of certain age to have the same weight, irrespective of their size as measured by height for example. It also includes such children who are underweight as a result of malnutrition in the past.

McLaren et al (1967) introduced a simple scoring system for classifying the severe forms only (satisfying Gomez criteria of weight $\leq 70\%$), based on all three methods of assessment viz. clinical, anthropometric and biochemical.

### McLAREN CLASSIFICATION

<table>
<thead>
<tr>
<th>Signs present</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema</td>
<td>3</td>
</tr>
<tr>
<td>Dermatosis</td>
<td>2</td>
</tr>
<tr>
<td>Oedema plus dermatosis</td>
<td>6</td>
</tr>
<tr>
<td>Hair change</td>
<td>1</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>1</td>
</tr>
</tbody>
</table>

* Serum albumin (Total serum proteins)

<table>
<thead>
<tr>
<th>(g/100 ml)</th>
<th>(g/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\leq 1.00$</td>
<td>$\leq 3.25$</td>
</tr>
<tr>
<td>1.00 - 1.49</td>
<td>(3.25 - 3.99)</td>
</tr>
<tr>
<td>1.50 - 1.99</td>
<td>(4.00 - 4.74)</td>
</tr>
<tr>
<td>2.00 - 2.49</td>
<td>(4.75 - 5.49)</td>
</tr>
<tr>
<td>2.50 - 3.19</td>
<td>(5.50 - 6.24)</td>
</tr>
<tr>
<td>3.00 - 3.49</td>
<td>(6.25 - 6.99)</td>
</tr>
<tr>
<td>3.50 - 3.99</td>
<td>(7.00 - 7.74)</td>
</tr>
<tr>
<td>$\geq 4.00$</td>
<td>($\geq 7.75$)</td>
</tr>
</tbody>
</table>

Score = Sum of points; 0-3 = marasmus;
4 - 8 = marasmus-kwashiorkor; 9 - 15 = kwashiorkor.

* Either serum albumin or total serum proteins were used for assessment.
This system has been used by a number of centres and is the only method available at present for a fairly precise and objective classification of the type of patients studied in hospitals. The problem of expressing chronicity and stage of disease however remains unsolved.

Some classifications have been designed to use measurements requiring only simple apparatus, avoiding the necessity for calculations and also the need to know the age of the child. These could be thus applicable under routine field conditions by unskilled personnel. Among these, Quaestick (Arnold, 1969) method uses the height and mid-arm circumference. Based on this classification children were divided into two broad categories, 'malnourished' and 'normal'.

The ratio of mid-arm circumference/height circumference was shown to be independent of age at least from 3 to 48 months and was similar in either sex (Kanwati and McLaren, 1970). Based on this ratio, the following classification has been proposed to detect cases of malnutrition.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 0.310</td>
<td>Nutritionally healthy</td>
</tr>
<tr>
<td>0.310 - 0.280</td>
<td>Mild PCM</td>
</tr>
<tr>
<td>0.279 - 0.250</td>
<td>Moderate PCM</td>
</tr>
<tr>
<td>&lt; 0.250</td>
<td>Severe PCM</td>
</tr>
</tbody>
</table>
However, it needs to be emphasized that the method is rough, should not be used for individual children and is meant to screen large numbers.

The classification that appeared in the 8th report of FAO/WHO Expert Committee (1971) is one that was originally prepared by the Wellcome Trust and is sometimes referred to as the 'Wellcome' classification.

**WELLCOME CLASSIFICATION**

<table>
<thead>
<tr>
<th></th>
<th>Body weight as % of standard*</th>
<th>Oedema</th>
<th>Deficit in weight for height**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight child</td>
<td>30-60</td>
<td>0</td>
<td>Minimal</td>
</tr>
<tr>
<td>Nutritional dwarfing</td>
<td>&lt; 60</td>
<td>0</td>
<td>Minimal</td>
</tr>
<tr>
<td>Marasmus</td>
<td>&lt; 60</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>80-60</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Marasmic-Kwashiorkor</td>
<td>&lt; 60</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

* Standard taken as 50th percentile of the Harvard values.

** Weight for height = \( \frac{\text{Weight of patient}}{\text{Height of normal subject}} \times 100 \)

'Wellcome' classification was probably the first in which an attempt was made to use weight/height as well as weight/age ratios and included a separate category of 'nutritional dwarfs'. However, it has some notable deficiencies. It confuses between the type and severity
of malnutrition. Diagnosis of marasmus, marasmic-
kuashiorkor, and kuashiorkor refer to differences in the
type of malnutrition and all are of similar severity.

Thus in this system kuashiorkor appears to be less severe
than the other two types as the body weight is 60-80% of
standard and not below 60%. Gradation of deficit in
weight for height by such terms used as 'Minimal' and
'+' can not be quantitated.

Nutrition Sub-Committee of the Indian Academy
of Pediatrics (1973) classified PCM into 4 grades using
50th percentile of Harvard growth standard as a
reference point.

CLASSIFICATION OF INDIAN ACADEMY OF PEDIATRICS

<table>
<thead>
<tr>
<th>Grade of malnutrition</th>
<th>Weight expressed as percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>of reference standards.</td>
</tr>
<tr>
<td>I</td>
<td>71 - 80%</td>
</tr>
<tr>
<td>II</td>
<td>61 - 70%</td>
</tr>
<tr>
<td>III</td>
<td>51 - 60%</td>
</tr>
<tr>
<td>IV</td>
<td>(&lt; 50%</td>
</tr>
</tbody>
</table>

Grade I and II are underweight and grade III and
IV correspond to marasmus. When nutritional oedema is
present, letter 'K' is suffixed to the grade denoting mal-
nutrition, eg, 1K; 2K etc. 1 K and 2 K will mean
kuashiorkor and grade 3 K and 4 K will correspond to
marasmic-kuashiorkor.
Waterlow and Hutigbauer (1974) published a classification based on weight and height, thus taking into account the effect of past as well as present malnutrition. The 'present' malnutrition was called 'wasting' as measured by loss of weight in relation to height, and 'past' malnutrition called 'stunting' was seen as low height for age ratio.

**WATERLOW AND HUTIGBAUER CLASSIFICATION**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Stunting (height for age)</th>
<th>Wasting (weight for height)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7 90%</td>
<td>7 0%</td>
</tr>
<tr>
<td>1</td>
<td>95-90%</td>
<td>90-80%</td>
</tr>
<tr>
<td>2</td>
<td>89-85%</td>
<td>80-70%</td>
</tr>
<tr>
<td>3</td>
<td>85%</td>
<td>70%</td>
</tr>
</tbody>
</table>

Waterlow maintained that weight/height was independent of age, basing his argument on two sets of data which were collected on children age between one and four years.

**MALNUTRITION, INFECTION AND IMMUNITY**

Sorimshau et al (1969) reported that the nutritional status was a critical determinant of susceptibility to infection. He supported his clinical impression by epidemiological data and experimental studies in laboratory animals.
Philips et al (1969) also found that children with PCM were unusually susceptible to severe infections and took longer time to combat such infections.

Ramalingamani and Ramalingamani (1973) observed that malnutrition and infection, singly and in combination, contributed significantly to morbidity and mortality of infants and children in the developing countries.

In another comprehensive study, Reddy et al (1979) concluded that nutrition, immunity and infection were closely linked. They showed that inadequate nutrition could alter the immunocompetence, thus increasing the susceptibility to infection, and infection in turn, adversely affected nutritional status.

**Defence Mechanisms in PCM:**

In defence against bacteria, viruses and other pathogens, several facets of immunocompetence come into play. Phagocytic activity and bactericidal competence of leucocytes constitute the first order of defence. In addition, two other types of immune mechanism, which operate against infection, are the humoral and cell mediated immunity. Also, there are other nonspecific defence factors such as lysozyme, complement and opsonins which play an important role in determining resistance to infection. Alterations in one or more of these mechanisms may be expected to increase susceptibility to infections.
FCM causes depression of several defence mechanisms.

Smythe et al (1971) demonstrated profound depletion of the thymolymphatic system and severe depression of cell mediated immunity in malnutrition.

Chandra (1972) noticed that antibody response to tetanus toxoid was adequate, but response to S. typhi vaccine was significantly reduced in malnourished children. He also reported depressed cell mediated immune response in FCM.

Selvaraj and Dhat (1973) and Seth et al (1972) showed that phagocytic and killing functions of leucocytes were decreased in children with FCM.

Edelman et al (1973) observed depressed inflammatory response and cell mediated immune response in FCM.

Reddy et al (1977) showed that both the cell mediated immune response and antibody response to bacterial antigens were impaired in children with severe FCM. However, the immunological responses were not altered in those with mild to moderate FCM as observed by authors.

Kumar et al (1978) observed depressed cell mediated immunity in children with FCM.

Puri et al (1980) reported that various parameters of cellular immune response were significantly depressed in severe FCM. However, the authors also
observed that humoral immunity was not altered in PCP except in the presence of infection, when there was some increase in IgG levels.

**COMPLEMENT SYSTEM:**

Complement is a system of factors occurring in normal serum which are activated characteristicly by antigen-antibody reaction and subsequently mediate a number of biologically significant consequences. It is now apparent that complement acts as the principal mediator of the inflammatory response and plays an essential role in host defences against infection.

According to McConnell and Lachmann (1976) and Pepys (1976), role of complement has advanced in recent years from being a collection of abstruse biochemical phenomenon to a system which has fundamental importance in immunogenetics and immunopathology.

**History:**

Pfeiffer (1894) demonstrated that the immune system of guinea pigs acquired the capacity to dissolve cholera bacteria (Pfeiffer's phenomenon).

Bordet (1896) repeated the experiment and found that Pfeiffer's phenomenon required two components of serum: a heat stable component (stable at 56°C for 30 minutes) that was present only in immune serum and a heat labile component present in immune as well as nonimmune sera. Bordet described the same phenomenon
in the serum of animals immunized with red blood cells of different species and called heat labile factor 'Alexin'. The term alexin was later replaced by the new term 'Complement' proposed by Schilling and Morgenroth (1899). These authors concluded that serum contained two substances: the interbody having two haptophore groups (analogous to immune body) and an addiment, which they named complement because it completed the antibody's immune response after it reacted with antigen.

By the 1920s there were 4, by the 1960s, 9 components were known (one of which had 3 subcomponents). Austen et al (1969) labelled the original system of 11 interdependent factors as the classical pathway of complement.

Gotsch et al (1971) and Osborn (1972) described a second major pathway of activation of complement, the alternative or properdin pathway. Authors also reported that this system consisted of at least 4 factors.

**Basic Precepts:**

Johnston and Stroud (1977) described the basic precepts of complement system:

1. Complement is a system of interacting proteins. The biologic functions of the system depend upon the interaction of individual components.

2. The components interact in an orderly, sequential fashion. This has been referred to as 'Cascade', in that activation of each component (except the first) depends upon activation of the prior component or components in the sequence.
3. Interaction occurs along two pathways:

The Classical Pathway—in which the components interact in the following order: Antigen—antibody C142356789, and the more recently discovered alternative or Properdin Pathway. In this alternative pathway the chain of reaction is: Activator (antibody) — Properdin system — C356789. Whether an antibody is required and what is the exact sequence of interaction of components in the alternative pathway is still not clearly understood.

4. The interaction of early acting components (C14235) is enzymatic in nature, so that "activation" refers to transformation of the components into an active enzyme. In contrast, the interaction between C5b, C6, C7, C8 and C9 is non-enzymatic through non-covalent, probably hydrophobic, bonds. In the case of C1, activation is a result of its interaction with antibody. Activation of C4, C2, C3, C5, as well as of factor B of the alternative pathway, is secondary to cleavage by a preceding component or components. This activation of early components generates an enzyme which fixes to the antigen—antibody complement complex and catalyses a reaction on the next component, whereas later acting components (C6 to C9) adsorb on to the complex or the underlying cell by an interaction which depends on a change in their configuration.
Sequence of Activation:

Johnston and Strobel (1977) summarized the sequence in which the components of the classical pathway and alternative pathway interacted. The interdigitation between classical and alternative pathways and the classical and functional by products of these reactions were also described (Fig. 1, 2).

The Biological Role of the Complement System:

Fust (1978/1979) inferred that the complement system played an essential role in a number of physiological processes participating in the defence mechanism of the organism and were mostly favourable. However, he emphasized that like other plasma enzyme systems, complement played a dual role. He expressed the opinion that events occurring during complement activation and the substances liberated during such activation could induce pathological processes. To substantiate his opinion, author quoted the example of complement playing an essential defensive role in the elimination of immune complexes (ICs) while it also caused tissue destruction.

Participation in Host Defence:

Dias da Silva et al (1967) and Shin et al (1969) expressed that complement was a dominant force in mediating inflammation, phagocytosis and cytolyisis. They showed that when the functional unit was activated, both C3a and C5a allowed histamine release from mast cells.
The Complement System

Classical pathway

(C-CMP) → C4a → C4b → C4c1c2 → C5a "C5a" 'Kinin' → C3

Requires Ca²⁺ & Mg²⁺

Alternative pathway

Properdin system

(F) C3G5b
Properdin Factor D Factor B
C3b ina & B1H
C3b amplification
C5a anaphylatoxin
Enhanced phagocytosis
Enhanced adherence
Binding to B cells (C3b, C5a)
C5a anaphylatoxin
Chemotaxis

Fig. 1: Sequence of activation of the complement components of the classical pathway and interaction with the properdin system.

C = Erythrocyte (could stand for any antigen 'Ag' e.g., bacterium, virus, tumour cell); A = Antibody (Ab) (of IgG or IgM classes only);
C-CMP = C - Carbohydrate - C-reactive protein; CI INA = CI inhibitor;
C3b ina = C3b inactivator; IgS = Immunoglobulins; EPS = Lipopolysaccharides.
**Fig. 2**: Interaction of components of the alternative (properdin) complement pathway; Ab = Antibody; Ag = Antigen.
and basophils. Besides, there were muscle contractions, an increase in capillary permeability and leucotaxis of neutrophils, eosinophils and mononuclear cells.

Gether (1972) reported a small molecular weight cleavage fragment (C3a?) from C5 which was responsible for release of neutrophils from bone marrow.

Complement forms a vital link in host resistance to infection. During infection with bacteria, parasites and yeasts, the processes of cytolysis and phagocytosis occur. Allison (1974) observed that with viral infections, cytolysis and phagocytosis did occur but in addition, complement also participated in the process of neutralization. Author described that this process either prevented the virion from entering into the target cell or that it interfered with the replication of virion inside the cell.

Strauss et al (1975) stressed that C3 activation might be important for initiating oxidative metabolism of the polymorphonuclear leucocytes and the release of lysosomal enzymes.

Kopeyan et al (1976) reported that by interaction with B type lymphocytes, C3b mediated the release of chemotactic factors from macrophages, B cell proliferation and formation of antibody.

Miller et al (1976) observed that neutralization of virus required either the deposition of C1, C4 and C3 or the fixation of C3 on to the virus. Depending on the
type of virus, activation and deposition of C3 could occur by the classical or alternative pathway. Antibody was not necessarily required in complement activation since some viruses could directly activate C1 by contact, as observed by the authors.

Spitzer (1977a) reported that besides providing for various elements of inflammation, complement was also involved in a direct attack on pathogenic agents by cytology, the entire functional unit being necessary for this purpose whether through classical or alternative pathway. Author also showed that by the deposition of C3b on the surface of the offending organism, complement system promoted phagocytosis by providing a contact point between the organism and phagocyte thus allowing internalization. This fact was further substantiated by Johnston and Stroud (1977). Johnston and Stroud (1977) summarized specific activities of the complement system in host defense against infection as follows:
## Activities of Complement in Host Defence Against Infection

<table>
<thead>
<tr>
<th>Components or Fragments</th>
<th>Functional activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1a, C1423</td>
<td>Virus neutralization.</td>
</tr>
<tr>
<td>C3a, C5a</td>
<td>&quot;Anaphylatoxin&quot; (Capillary dilatation)</td>
</tr>
<tr>
<td>C3 &amp; C5 fragments</td>
<td>Chemotaxis of PMNs,</td>
</tr>
<tr>
<td>C567</td>
<td>Monocytes, eosinophils</td>
</tr>
<tr>
<td>C3b</td>
<td>Opsonization</td>
</tr>
<tr>
<td>C3b, C3d</td>
<td>Enhanced induction of antibody formation.</td>
</tr>
<tr>
<td>C3b</td>
<td>Stimulation of B-cell lymphokine production.</td>
</tr>
<tr>
<td>C3 Cleavage product</td>
<td>Induction of granulocytosis</td>
</tr>
<tr>
<td>C5</td>
<td>Opsonization of fungi</td>
</tr>
<tr>
<td>C1-6 (?additional components)</td>
<td>Endotoxin inactivation</td>
</tr>
<tr>
<td>C1-9</td>
<td>Lysis of viruses, virus infected cells, tumour cells, mycoplasma, protozoa, spirochetes and bacteria.</td>
</tr>
</tbody>
</table>

Fust (1978/1979) suggested that alternative pathway represented the first defence line against bacterial infections; it was capable of reacting with bacteria, opsonizing them and supporting their elimination before the specific antibody response would start.
The Role of the Complement System in the Elimination of

Immune Complexes (ICs):

There are different complement mediated processes which cooperate in IC elimination.

According to Gigli et al (1968) and Ruddy et al (1972), the ICs, bearing C3b on surface, were capable of binding to the C3b receptors of polymorphonuclear leukocytes and also to the cells of the mononuclear phagocyte system. Thus ICs were finally phagocytosed.

Miller et al (1975) reported that C3b in the immune complex could change the conformation of the complex itself. As a result, large complexes were split into the smaller ones. These smaller complexes were unable to deposit in the tissues and got ultimately detoxified.

METHODS OF EVALUATION OF COMPLEMENT SYSTEM:

1. Functional Assessment:

Functional assessment of the activity of the complement system is made by measuring the lysis of antibody coated sheep erythrocytes (for total haemolytic complement) or unabsorbed rabbit erythrocytes (for alternative pathway activity) by normal human serum.

1) Total Haemolytic Complement (CH$_{50}$):
The technique of determination of total haemolytic complement (CH$_{50}$) was originated by Mayer (1961). He observed that testing for total haemolytic complement served as a useful screening procedure for most of the
diseases of the complement system. This assay depended upon the ability of all 9 classical pathway components to interact and lyse antibody coated erythrocytes. Author concluded that the dilution of serum which lysed 50 percent of the cells, determined the end point and the reciprocal of that dilution was the $C_{H50}$ or "complement hemolysis of 50% of cells."

Spitzer (1977b) reported that some of the components might be reduced significantly in amount without causing a noticeable deviation in the $C_{H50}$. Thus with C3, for example, it took nearly a 50% reduction to decrease the $C_{H50}$ since C3 was normally present in large quantities in serum. In view of this major disadvantage of this screening test, author inferred that one could not place too much emphasis on this single assay.

Interestingly, Johnston (1979) drew attention and reported that in the congenital deficiencies of one or more classical pathway components, the $C_{H50}$ value would be zero or almost so; values in acquired deficiencies would vary with the severity of the underlying disorder. Author also strongly emphasized that this procedure should be available as a screening test to every physician.

2) Alternative Pathway Activity:

Flatta-Mills and Ishizaka (1974) observed that unsensitized rabbit erythrocyte (RBC) activated the
alternative pathway of complement in normal human serum. Hence authors used the lysis of RBC to assess the functional activity of the alternative pathway components including (C3-C9) and it was expressed as \( \text{CF}_{50} \).

3) **Functional or Immuno-haemolytic Assay for the Different Components of Classical and Alternative Pathway**

Fust (1978/1979) described the use of functional (immuno-haemolytic) assay for the assessment of different components of classical and alternative pathway. In the procedure, great excesses of the preceding components were added to sensitized sheep erythrocytes. Then serial dilutions of the serum (under test) were added to the system to serve as the only source of complement component to be tested. Finally the latter components were added. Author showed that the concentration of tested component could be calculated from the percentage haemolysis of RBCs and the corresponding serum dilution. Titre of the component was expressed in \( \text{CH}_{63}/\text{ml} \) units (\( \text{CH}_{63} \) being the complement quantity causing lysis of 63% of the erythrocytes).

II- **Immunochemical Assessment**

The immunochemical technique is an important measure of quantitative assessment of various individual complement components in the serum.
The double diffusion technique as derived by Ouchterlony (1948) is a qualitative technique, used to detect the presence of antigens or antibody in a test solution and to show antigenic cross reactivity. The author described that when antigen and antibody were placed in wells cut in a gel and allowed to diffuse, visible precipitin lines were formed at the zone of equivalence. By a system of serial dilution of test samples, an approximate concentration or titre could also be derived, as reported by the author.

Grabar and Williams (1953) described the technique of immunoelectrophoresis. Immunelectrophoresis combined the advantage of serial electrophoretic separation of proteins and the immunological discrimination of double diffusion.

Mancini et al (1965) described Single Radial Immunodiffusion, as a technique for quantitative estimation of proteins (antigens). Authors showed that the antigen diffused radially from the point of application into an antibody containing gel and a circular precipitate (ring) was formed at the zone of equivalence. Keeping antibody concentration and gel thickness constant; the area covered by precipitin ring was proportional to the concentration of antigen. In the original method, authors allowed the antigens to diffuse at room temperature until the precipitin rings stopped growing in size.
Fahy and McKelvey (1965) modified Mancini's technique of Single Radial Immuno-diffusion. They reported that the readings could be taken after a fixed time viz. 18-20 hours; giving rise to only minute differences in the results.

Laurell (1966) described the technique Rocket Immune-electrophoresis, a simple, quick and reproducible method for determination of a single protein in a protein mixture using number of samples simultaneously. Author applied diluted samples in wells side by side in a layer of agarose gel containing a nonspecific antisemum. The identification of the protein was given by the rocket-shaped precipitate formed and quantification was done by measuring the height of the precipitate rocket or the area under it.

III- Demonstration of Complement Activation Products:

During last few years, direct methods for the demonstration of complement activation or breakdown products have become popular.

Lachmann and Coombs (1963) found that the titre of immunoglobulins in serum, as antibody to reacted C3 and C4, was a measure of the extent of, in vivo, complement activation.

Thompson (1977) described a simple and reliable technique, known as 'Two Dimensional or Crossed Immune-electrophoresis'. Author based this
test on the principle that both C3 and its activated fragment shared the same antigenic determinant but had different electrophoretic mobilities.

IV- Miscellaneous Method:

Fuest (1978/1979) observed that deposition of complement components in various parts of renal glomeruli was of special importance in the diagnostics of certain renal diseases. Author also noticed that these deposits were made visible by immunofluorescence.

Simplified Scheme for Evaluation of Complement:

Spitzer (1977b) gave a simplified scheme for evaluation of complement associated disorders by three screening tests including CH50, C3 and C6 assays (Fig.13).

PCM and Complement:

Smythe et al (1971) were the first to call attention to altered total hemolytic complement (CH50) activity in infants with PCM. Estimation of hemolytic complement by these authors showed that 61% of infants with PCM had values well below 1/64 and 39% had values within normal limits, whereas the controls were consistently within a range of 1/128 to 1/512. These differences were found to be statistically significant.

Chandra (1972) first estimated the serum levels of complement component C3 in malnourished children by Mancini's single radial immune-diffusion
Fig. 3: Simplified scheme for evaluation of complement-associated disorders; NI, Normal; *, can be normal; †, differentiated by assays for C1, factor B, and properdin or properdin convertase.
Serum levels of C3 were significantly lower in the malnourished children (95 mg% + 33 S.D.) than in the control subjects (126 mg% + 31 S.D.). It was also suggested that this could well be the result of reduced synthesis by the liver cells.

Sirisinha et al (1973) studied the serum levels of complement proteins C1q, C1s, C3, C4, C5, C6, G8, G9 and C3 proactivator (C3 P.A.) in twenty children with PGM on admission and at intervals thereafter during different dietary treatments and compared the results with those in nineteen normal children of the same age in the same geographical area. A majority of patients were judged to be infected on admission and were placed on antibiotic therapy. The authors also found that the serum levels of all the complement proteins except C4 were markedly lower in malnourished children than in the normal children, and children with kwashiorkor had lower complement levels than those children suffering from marasmus. Admission levels of C of the 9 components (except C4) were slightly lower in the severely infected compared with the mildly infected patients as observed by the authors. Further, the difference between the two groups was less pronounced on day 6, when infection seemed to be under control. However, the differences in the complement levels between severely infected and mildly infected patients were smaller than those between the malnourished and the normal children. These results
suggested that the poor diet associated with impaired synthesis of complement components and to a lesser extent, infections led to reduced serum complement levels in untreated PCM children. During follow up the quality of dietary protein and the calorie intake had a pronounced influence on the repair of the complement system, the best response was obtained by high calorie (175 C/kg/day) and high protein diet (4 g/kg/day). The levels of most complement components during treatment rose to above normal values. Mechanism of this 'overshoot' or 'rebound' should be due to accelerated synthesis after increased complement consumption in vivo as suggested by authors. They also suggested that, in addition to complement consumption, correction of impaired synthesis could result in complement 'rebound'.

In view of above observations, Chandra (1975) again subjected 35 children, aged 6 months to 4 years, having PCM to complement study and matched with 20 healthy controls. He observed that in 12 children there was clinical and microbiological or radiological evidence of systemic infection. These were treated with appropriate antibiotics. Three to eight weeks later a second sample of blood was drawn from 10 children, available for re-examination and no longer undernourished. He noticed that total haemolytic complement and C3 concentration were significantly decreased in
malmoured children than that of healthy controls.
There was also a significant positive correlation
between C3 concentration and CH50 activity (r = 0.7131).
Author observed a greater reduction in complement levels
in malmoured children with infection compared with
uninfected ones. However, in nutritionally normal
subjects, infection was associated with high C3 levels.
Author expressed the opinion that reversible but pro-
found disturbance of complement seen in infected under-
nourished patients could be the result of at least two
factors. One, antibody synthesis and cell division
might get priority over complement synthesis in the
face of limited nutrient resources of the host.
Secondly, infection might be associated with complement
consumption. Presence of second phenomenon, operating
in these patients was supported by the fact that
electrophoretically altered form of C3 in 14 cases
and raised levels of immunoglobulin were detected
in most of the cases. Finally the author suggested
that reduced complement function in malnutrition was
the combined result of impaired synthesis, complement
activation in vivo, change in plasma volume, protein
losing gastroenteropathy, and that it might contribute
to an increased susceptibility to infection in under-
nourished individuals.

Weman et al (1975) studied 76 malmoured
Chennai children, aged 6 months to 6 years, and 41
age matched controls. Cases were divided into three groups: 

Group I - Severely malnourished (3%) 
children whose weights were 51-60% of 50th percentile 
of Harvard standard and/or serum albumin levels below 
2.5 g/dL. These children formed two subgroups - 
Kwashiorkor (22) and marasmus (11). Group II - Moderately 
malnourished, included 43 children whose weights were 
61-80% of standard and serum albumin levels greater than 
2.5 g/dL and had minor skin and hair abnormalities. Lastly 
Group III - Control group consisted of children whose 
weights were ≥70% of standard, had normal serum albumin 
levels and were free of chemical signs of malnutrition 
or obvious infection except for pyoderma in a few cases. 
However, intestinal parasites were found in most of these 
cases. Authors noticed that levels of complement C3 were 
significantly reduced in the severely malnourished group 
as compared to the other two groups. Mean C3 levels in 
group II and III were slightly reduced but in a low 
normal range when compared to normal American children. 
In group I, children with kwashiorkor had lower C3 levels 
(mean 56.5 mg/100 ml) as compared to children suffering 
from marasmus (mean 71.7 mg/100 ml). After 2 weeks of 
nutritional therapy mean C3 level in group I children 
with kwashiorkor increased to 75.2 mg/100 ml. C4 
levels were found to be normal in all 3 groups. Author 
explained that decreased C3 levels could be due to 
diminished protein synthesis by the liver as suggested
by a good correlation between the degree of C3 depletion and severity of depletion of other proteins. They also did not rule out the possibility of accelerated consumption as a result of infection occurring in these cases.

Normal C4 level suggested that the alternative pathway of complement was activated in malnutrition probably by bacteria and their endotoxins which led to breakdown of C3 and later components without affecting C1, C2 and C4.

Using the haemolytic complement (CH50) assay, Suskind et al (1976) evaluated the complement system of 28 children with severe PNM during their hospital admission and recovery. Children were classified clinically as having marasmus (M), marasmus-Kwashiorkor (MK), and Kwashiorkor (K) based on the scoring system of McLaren et al (1967). The mean CH50 activity in children with kwashiorkor was significantly less on hospital days 1 and 4 than in 17 healthy control subjects. On day 8 it rose to normal, and by day 50, it was significantly higher than controls. The mean CH50 titre of 16 well nourished febrile children was, in contrast to that of untreated PNM, significantly greater than in the healthy controls. Therefore it was unlikely that fever present in many PNM children, lowered their CH50 activity. Among children with PNM, 11 (40%) had detectable serum anticomplementary (AC) activity in their serum on either day 1 or 4. Significantly, the CH50 titre in a PNM serum correlated inversely with the amount of AC activity in the serum.
These results indicated that, in children with PCM, complement system was compromised functionally, and that its repair coincided with the intake of adequate diet. Further, presence of AC activity provided a possible explanation for depressed complement activity in some untreated PCM children.

Complement components C1−C9 were also estimated in children with protein-calorie malnutrition by Olusi et al (1976). Concentrations of C1q, C1r, C3, C6 and C9 were significantly lower in children with PCM, than in age and sex matched control children as observed by the authors. Children with marasmus tended to have higher values of these complement components than children with kwashiorkor. Complement C3 and C9 were the most severely affected by malnutrition and it would appear from the study, that more severe the degree of malnutrition, as judged by clinical examination and serum transferrin concentration, greater was the reduction in the serum concentration of C3 and C9. It was observed that the serum concentrations of C3 and C9 were lower in kwashiorkor and marasmic children with infections than in children without infections. There was no correlation between C3 and IgG concentrations as reported by the authors. It was suggested that probable responsible factors for reduced complement activity in malnutrition were reduced protein synthesis and increased utilization due to concomitant infections.
It was significant to observe in this series that there was no change in C4 concentration in children with malnutrition. It would appear that C4 was synthesized by the same cells responsible for the production of IgG and hence that there was a preferential synthesis of C4 and IgG in children with PEM. C5 was the only complement component which was significantly higher in malnourished children than in normal children, thus suggesting that this complement component was an acute phase protein. During refeeding, C3 was the first complement component to show a significant rise in concentration; this was followed by C9 and then C6. There was no change in C4 concentration while the levels of C5 fell. A conclusion drawn from these observations was that, of all of the complement components, C3 was the most sensitive index of nutritional status.

Kielmann et al (1976) carried out first ever study in nonhospitalized pre-school children in nine villages of the former Narangwal Rural Health Research Centre in Ludhiana District, Punjab. In these villages, all children up to 3 years of age routinely received curative and preventive medical facilities besides food supplement. Authors divided the children into 3 groups based on weight for age. These groups corresponded approximately to 80% or higher, 60% to 79% and less than 60% of the Harvard median, respectively. The children had significantly lower complement levels
(for all the three groups) as compared to those of reference population of identical age distribution. Children in the lowest weight for age group had less than 50% and those in the two higher nutritional groups had between 60% and 70% of the complement levels as compared to the reference population. Complement C3 levels were also positively correlated with several other anthropometric indices (weight–chest circumference and arm circumference for age) as observed by the authors.

Spitzer (1977a) mentioned that in patients with malnutrition, a consideration of failure of synthesis of C3 might be entertained. Decreased C3 levels could be used for diagnosis and also for monitoring during follow up.

Naller et al. (1978) measured the plasma levels of complement haemolytic activity (CH₅₀), of some complement components and of C3d, a C3 break–down product, in 89 African children with various types of PEM including kwashiorkor, before and during recovery and compared them with two control groups, each consisting of ten age matched children and having a weight–age ratio of 790% of the Harvard standard. One of the control group was suffering from infection at the time of admission and the other had none. A significant decrease of CH₅₀, C3, C9 and factor B was observed in PEM. The decrease of CH₅₀, C3 and C9 appeared to be correlated with the severity of PEM, which was not the case for factor B.
On the other hand, levels of C4, C3 and C1 - Inactivator fell within normal range. Increased plasma levels of C3d with higher C3d/C3 ratio were also found in PEM patients as compared to non-malnourished infected patients and to normal non-infected children. Serial measurements done during the recovery of PEM indicated a progressive normalization of all complement values, as well as a decrease of C3d/C3 ratio. Presenting their conclusions authors thought that two mechanisms could possibly be involved in the impairment of complement system in PEM: (1) a decreased synthesis of at least C3 and C9, as suggested by a significant correlation of C3 and C9 levels with those of serum albumin and cholinesterase; (2) an increased catabolism of C3, possibly due to an activation of the alternative complement pathway, as suggested by the increased levels of C3d and decreased level of factor B both of which were significantly correlated with C3 levels but not with albumin levels. Again C4 levels were normal as observed by authors.

Kellmann and Curcio (1979) observed C3 complement levels in 53 rural pre-school children of North India. They related C3 complement levels to various parameters of nutritional status and past episodes of infections. All children were normally active and free from intercurrent infections. Mean complement levels were 25% lower than those found in an age-matched European
reference population. Low complement (C3) levels were associated mainly with children who were both stunted and wasted, as well as with those who had experienced frequent parasitic skin infections in the past.

According to Johnston (1979) patients with malnutrition could have significant depletion of complement components and functional activity of complement. Although synthesis of components was depressed in malnutrition, serum from some patients also appeared to contain immune complexes which could accelerate depletion.

In a recent study Jagadeesan and Reddy (1979) reported that total haemolytic complement (CH50) as well as C3 levels were significantly decreased in children with kwashiorkor (some of these had associated infections) and returned to normal after 3-4 weeks of treatment with protein and calories. In marasmic children, though the total complement activity was not significantly altered, C3 levels were reduced. However, neither CH50 nor C3 levels were found to be altered in mild to moderate protein-energy malnutrition (weight between 60-80% of standard). Reduction in serum complement activity could be one of the factors responsible for the frequent occurrence of infections in children with severe PEM as suggested by authors. Their study also indicated that immune status was not affected by milder degrees of PEM.