REVIEW OF LITERATURE
"Earth has two powers; the power of population is infinitely greater than the power in the earth to produce subsistance for man".

-Malthus Robert

The disparity in population growth and food production is all the more marked in the developing world with the result that malnutrition, particularly in the young, is rampant. An inadequate dietary intake of calories and proteins of good quality is widespread; serious nutritional problems in technically under-developed areas have devastating effects particularly on infants and children. Therefore, malnutrition is the greatest material problem and challenge which faces the mankind today. Tremendous amount of manpower resources, research and effort has gone into its proper understanding and eradication and voluminous literature has accumulated on the subject in recent times.

In the light of recommendation's by International Union of Nutritional Sciences(IUNS),
malnutrition may be defined as any deviation from normal nutrition. Literally the term malnutrition means bad nutrition (Malas-bad) and is generally considered to be synonym of undernutrition. Romans called malnutrition as 'Macies' 'Macilantia', 'Graciletes', and 'Machitudo!', Greeks labelled it as 'Micromunmm Mercurialis' as early as in 1683. According to Smellie (1954), the term 'Marasmus' was first introduced in 1600 by Seraino to describe the condition of infants who were suffering essentially from starvation. Marasmus is derived from a Greek word meaning 'to waste'. Pamell (1653) referred to it for the first time in the English literature. Harris described it as 'Atrophia Verminosa' and Parrot (1874) labelled it as 'Athrepsia' Cachexia. Denutrition, inanition and infantile atrophy are some of the other terms used for marasmus. The term 'starchy food dystrophy' was used by Czerny and Keller (1906) for what is now commonly known as marasmus.

The term 'Kwashiorkor' was given to native children of a certain section of Africa, in whose dialect it means 'the disease of the deposed baby when the next
one is born\. It is comparatively a new disease, being first introduced into the medical literature in 1953 by Dr. C.D. Williams, a Jamaican paediatrician working in African Gold Coast. It was characterized by oedema, dermatosis, diarrhoea and fatty liver, and was thought to be nutritional in origin but distinct from pellagra. Since then, this condition has attracted a great deal of attention from workers in different parts of the world and has been described under such different names as 'malignant malnutrition', nutritional dystrophy; nutritional oedema syndrome, mehlnahrschaden, sugar baby syndrome, pluricarential, culebrills etc.

In the first half of the present century, the most commonly described nutritional deficiencies were those of vitamins such as in scurvy, pellagra and rickets and of minerals such as iron and iodine. However, nutritionists and public health workers are becoming increasingly aware of the fact that the most serious and urgent nutritional deficiency problem in the world today is that of protein-calorie malnutrition (Jelliffe, 1963; Behar, 1968). Protein-calorie malnutrition (PCM) has been defined as a range of pathological conditions arising
from the coincident lack, in varying proportion's of proteins and calories, occurring most frequently in infants and young children and commonly associated with infection's (FAO/WHO, 1973). Protein-calorie malnutrition of various degree is the most prevalent disease of nutritional deficiency in the world (Scrimshaw and Behar, 1959). No other disease compares in importance with PCM in the field of nutrition and public health in general. This form of malnutrition is highly prevalent primarily in young children, although older children and adults of both sexes are not spared (Holmes and Trowell, 1948; Viteri and Gulino, 1964). Few children survive the most severe forms of PCM unless they receive medical attention; even then, mortality is still high. The disease in its less severe form constitutes a serious handicap and may have permanent effects on the growth and development of the child, if it continues over a long period or if it is present at a very early age.

Dr. J.M. Bengoa has assessed the current evidence of the prevalence of PCM in the world (Bengoa, 1972, 1974). He emphasized that any estimate can only be an approximation. The joint FAO/WHO Expert Committee on Nutrition
(1971) similarly found it difficult to obtain even a rough estimate of the total number of malnourished children in the world and had made suggestions for improving the collection of prevalence data. Using the data provided by a reasonably large scale surveys carried out between 1963 and 1972, Bengoa (1974) estimated that in Latin America the proportion of children with either moderate or severe malnutrition is of the order of 19%, in Africa of 26% and in Asia of 31%. According to W.H.O. estimates, it appears that in world as a whole about 10 million children suffer from severe malnutrition (Bengoa, 1974). Another analysis of 101 community surveys conducted in 59 developing countries during the years 1961-1971 revealed that not less 100 million children below 5 years of age are affected by moderate to severe PCUH (Udani, 1977).

The community surveys conducted by various countries have indicated that 0.3 to 1.6% of the preschool children suffer from Kwashiorkor whereas 1.0 to 6.8% suffer from marasmus (Bengoa, 1970). A survey conducted by Dean (1959), Thompson (1960) and Dean (1961) in Malaysia indicated the incidence of Kwashiorkor as 6% in 1-4 years old children and in one district as many
as 75th of pre-school children showed signs of Kwashiorkor. Jelliffe and Jelliffe (1960, 1961) showed that 3-16% of Haitian children in the age group of 1-3 years were suffering from Kwashiorkor. Out of 1206 paediatric admissions in Medical College Hospital, Jabalpur (India), 413 (34.2%) had 3rd degree of PCP (Sukherjee et al, 1969). Ghai et al (1970), conducted a survey of 20 villages in the Ballabgarh block in Haryana (India) covering 3029 pre-school children and reported the incidence of Kwashiorkor in 0.9% and that of marasmus in 1.7% children. Gopalan and Vijayaraghavan (1971) have estimated that in India 2-3% of all the pre-school children suffer from frank PCP. Out of this Kwashiorkor accounts for 0.4 to 0.9% whereas marasmus accounts for 1-2% (Shah, 1975). The majority of the pre-school children suffering from PCP have mild to moderate grades of malnutrition as judged by the growth status (Reddy and Sammi, 1976). However, a small percentage (1-2%) of the children show signs and symptoms of severe PCP (marasmus of Kwashiorkor).

Protein-calorie malnutrition is perhaps the principal factor responsible either directly and/or indirectly for a considerable loss of child...
population in the developing world. Inter American investigations of mortality in children revealed that nutritional deficiency was an associated cause of 60.9% deaths from infectious diseases in children in the age group of 1-5 years, as well as 37.2% deaths from all other causes (Puffer and Seraino, 1973). However, many cases of mild to moderate malnutrition still remain undiagnosed. Reddy (1975 a) has reported a mortality rate of 10-50% in Indian malnourished children and in fact 12.4% of marasmic and 11.5% of Kwashiorkor children died. However, Ghai (1975) observed 14% mortality among the patients admitted with PCM. According to a report from WHO/UNICEF (1978), poor nutrition alone is responsible for half the deaths of children in the developing countries.

THE CLINICAL SPECTRUM OF PCM

A number of terms have been used in the past (and some are still in use) to describe the various pathological conditions that are now recognized to be only variants of PCM. The term PCM which was introduced by Jelliffe (1959) is preferred by most authors and was
adopted by the Joint FAO/WHO Expert Committee on Nutrition (1971). The term has the great advantage of bringing forward the main cause while avoiding any emphasis on clinical signs and symptoms. While it is true that PCM covers a wide spectrum of pathological conditions, the aetiology of entire spectrum of infant and child malnutrition is not fully reflected in the term PCM since not only the deficiency of protein and energy but also the deficiencies of other specific essential nutrients and infections are involved (FAO/WHO, 1971). Thus the phrase PCM refers to a spectrum of clinical disorders caused by various degrees of deficiency and additional physiological insults and stresses. The condition may vary in severity and in clinical manifestations, the ultimate pathological states at the two extremes being nutritional marasmus and Kwashiorkor (Waterlow, 1948; Waterlow et al, 1960). In practice, a large proportion of PCM cases occupy an intermediate position and are referred to as marasmic Kwashiorkor. The manifestations of severe PCM vary widely according to the nature of the causative factors, the time for which they operate, and the age of the patient. Moreover, a child with nutritional marasmus may develop marasmic Kwashiorkor
FIG. 1 SCHEMATIC REPRESENTATION OF THE NATURAL EVOLUTION OF DIFFERENT FORMS OF PEM
and a child with marasmic Kwashiorkor may present a picture of nutritional marasmus after oedema subsides.

The development of complete spectrum of PCM from apparent normality is shown in Fig.1. Time is the horizontal axis of a triangle whose asymmetrical shape portrays the relatively rapid development of metabolic disorder which can result in classical Kwashiorkor, in contrast to the much slower process of body wasting to the extreme limits of classical nutritional marasmus (Scrimshaw and Behar, 1961; Viteri et al, 1964; Viteri, 1981). The much more complex route followed by most children towards PCM is illustrated hypothetically by the interrupted lines. In these circumstances malnutrition does not develop under the steady primary proteins and calorie deficiency, but results from a complex, ever fluctuating mixture of the two deficiencies.

This nosological framework has been gradually expanded so that currently five clinical syndromes are grouped together as overlapping manifestations of PCM. This classification is based on a knowledge of patient's age, weight, height and presence or absence of oedema.
It has been recommended for general use by the Joint FAO/WHO Expert Committee on Nutrition (1971). The classification is tabulated below (Table 1).

**Table 1**

Simplified classification of PCM**

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Body weight as % of standard*</th>
<th>Oedema</th>
<th>Deficit in weight for height**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under weight child</td>
<td>80-60</td>
<td>0</td>
<td>Minimal</td>
</tr>
<tr>
<td>Nutritional Dwarfism</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>

**Legends to Table 1:**

*Standard taken as 50th percentile of Boston values

**Weight for height: \( \frac{\text{weight of patient}}{\text{weight of the normal subjects of the same weight}} \)


Dugdal, A.E. (1971)

Since every PCM is made up of a spectrum of conditions, the two extremes—starvation marasmus (nutritional marasmus) and the sugar baby variant of Kwashiorkor—comprise separate clinical syndrome (Waterlow, 1948). While one may lead to another yet clinically they are well recognizable entities. The major query from the point of view of prevention and management is whether the two syndromes are two different diseases with entirely different dietary etiology or two facets of one and the same disease process. It has been postulated that Kwashiorkor and marasmus are two facets of the same disease process. No difference has been found between the diets that produce Kwashiorkor or marasmus in pre-school children (Gopalan and Narasinga Rao, 1971). Therefore, the growth retardation of marasmus may be an adaptation to the stress of inadequate calories and protein, and metabolic processes including liver functions are well preserved. Kwashiorkor, on the other hand, is a dysadaptation to protein and calorie deficiency that is characterized by the oedema and metabolic changes (Jaya Rao, 1974).
Nutritional marasmus is principally due to a diet very low in both protein and calories (so called prolonged balanced starvation). While it can occur at all ages, including adulthood, it is seen most commonly in the first year of life - in contrast to kwashiorkor with its main impact on the age group of 1-3 years frequently as a result of early cessation of breast feeding and attempted artificial feeding with very dilute milk. It is also associated often with infective diarrhoea and sometimes with tuberculosis. A late form of childhood nutritional marasmus can occur in the pre-school period as a result of continuing grossly defective diet, or of prolonged unsupplemented breast-feeding (breast starvation). The incidence of nutritional marasmus, associated with diarrhoeal diseases, is on the increase in artificially fed infants in towns in developing regions of the world (Jelliffe, 1962; Cronje et al, 1963; Graham and Morales, 1963; McLaren, 1966).

PCM differs from most of the disease in that it is neither a response to an invading organism nor an endogenous breakdown of an internal mechanism or structure. It is a series of responses to an environment
of deprivation. The first one is of adaptation; only later, if the deprivation persists or becomes more severe, the physiological and behavioural adaptation become a maladaptation and eventually a breakdown occurs which is associated with overt clinical signs and symptoms.

In recent years it has been customary to use the term 'protein-energy malnutrition' (PEM) to describe a group or spectrum of diseases that often affect children living in poor communities in most developing countries. The expression suggests important concepts. These are that inadequate intakes of energy or protein, or both, are causative factors and that there is a continuous connection in terms of biochemistry and physiology between the extreme forms of PEM. Moreover, the term PEM is a general one, including disorders that may be considered to differ in degree (mild, moderate, severe) and in type for the severe degree (marasmus and Kwashiorkor). At the 6th meeting of the FAO/WHO joint expert committee on Nutrition (1962), in its revision of the International Classification of Diseases, it was proposed that a main group of nutritional diseases should
be classified as 'Hypoalimentation', and that one sub-group should consist of PEM, the two forms of which are (1) Kwashiorkor including marasmic Kwashiorkor and (2) marasmus.

For comprehensive clinical descriptions of the two syndromes, the reader may refer to Alleyne et al (1977), but for the present it will be sufficient to use a series of internationally accepted definitions suggested by a Wellcome Trust Working Party (Lancet, 1970). The crucial diagnostic features are the degree of body wasting and the presence of oedema. Thus, when Harvard weight standards (Stuart and Stevenson, 1959) are used, children of 60-80% expected weight-for-age are called cases of Kwashiorkor if oedema is present. Below 60% the diagnosis is marasmic Kwashiorkor if they have oedema or marasmus if oedema is absent. These definitions have the merit of simplicity but they do not, in any way, distinguish causes in the same manner that the term PEM quite specifically suggests a dietary cause.

THE AETIOLOGY OF PEM

The characterization of PEM in pre-school
children more than 30 years ago led to its recognition as a scourge of underdeveloped societies (Scrimshaw and Behar, 1959) and result of interaction of several factors among which two are more or less directly responsible for the disease and act synergistically. They are, (1) a quantitatively insufficient and qualitatively inadequate dietary intake, and (2) infectious processes such as gastrointestinal and respiratory infections and other infectious diseases of childhood (Behar and Scrimshaw, 1960). It was advocated that underfeeding, unbalanced diet, and recurrent intestinal and parenteral infections were important factors in the aetiology of PEM (Griffith and Mitchell, 1938; Marriott and Jean, 1941). The latter workers suggested that different forms of malnutrition were essentially and merely the stages of the same disease. They categorized different forms of malnutrition according to weight loss and used the term 'Athrapsia' or marasmus for infants having more than 40% weight loss. This concept was supported by Krause (1957).

Zoborosky (1943) described malnutrition as
a loose term applied to a variety of nutritional disorders with signs of defective growth and strength. He suggested that marasmus is an extreme stage of malnutrition resulting from prolonged partial starvation, protracted malnutrition, persistent indigestion and repeated attacks of enteritis. Nelson (1964) observed that besides infections and allergy to food, factors like syphilis, tuberculosis, metabolic and congenital malformations, prematurity and psychological disorders are the other important causes of marasmus. Pinklestein (1953) studied the problem under the head 'decomposition' and blamed sugar and salts for the injurious effects on intestinal mucosa which led to fall in the secretions resulting from the bacterial overgrowth, mal-absorption and fermentation of sugars. The role of mal-absorption and infection in malnutrition has also been stressed by Ghai (1977).

Protein-energy malnutrition is not a clear-cut deficiency state of one ingredient (Trowell et al, 1954; Gomez et al, 1957; Jalliffe, 1959; Waterlow et al, 1960; Viteri et al, 1968). There has been a tendency to over-emphasize the importance of either protein or energy
deficiency alone, whereas in fact the two deficiencies almost always occur together. It may also consist of specific deficiencies of essential nutrients such as vitamins and minerals. Many other ecological factors have been implicated, including environment, local resources and land use, duration of breast feeding and cultural factors (Gomez et al, 1957; Collis et al, 1962).

Failure to achieve growth potential in a child can be attributed to an insufficient or improper intake of nutrients, malabsorption and certain ecological factors. Recently, a clear concept of the significance of various forms of PEM has been obtained through interpretation of data on age, height, weight, food consumption, occurrence of infectious diseases, and childhood mortality (Bengo, 1968; Puffer and Seraino, 1973; Goldsmith, 1974). Information on PEM has resulted from long-term prospective studies of rural population in Africa and Latin America (McGregor et al, 1966; Craviato et al, 1966; Mata et al, 1967). It has become evident that malnutrition is an ecological problem.

In many parts of the world, starvation is
related to national poverty, famine, wars, and unequal distribution of food, which are beyond the control of the individual family. While poverty is widespread in the tropics and subtropics, improper management of the micro-environment at the family level undoubtedly plays a more significant role in the causation of malnutrition. The limited economic resources are often further strained by large family size, and by indulgence in social ills of the society. Thus, it is widely established that the higher the birth order of a child, the more is his nutritional status likely to be compromised. Another feature of underprivileged population is the close interval between the birth of the successive siblings. The arrival of a new member usually means that an elder sibling would be further deprived of nutrition and care. In fact, 'Kwashiorkor' in African tribal language means 'the disease of the deposed baby when the next one is born'.

Breast starvation was the term used by Housedon (1950) when the breast milk was just sufficient for a first few weeks and thereafter use of artificial milk, generally contaminated with microbes, resulted in diarrhoea and further loss of weight. There are conflicting
claims about the role of breast feeding in the development of marasmus or kwashiorkor. Gopalan and Ramalingaswamy (1955) observed that prolonged breast feeding adversely affected the growth of the infant. However, Sheldon (1936) and Bhattacharya and Chaudhury (1962a) claimed that breast feeding protected the child against marasmus or kwashiorkor.

The declining trend in breast feeding is aggravated by inappropriate commercial advertising which serves to pressurize the young mothers into accepting artificial cow's milk formulae as a modern, convenient, superior, and desirable method of feeding. Because of high costs involved in artificial feeding, a large number of impoverished mothers resort to administering grossly diluted feeds to their infants. Moreover, in the tropics and sub-tropics, low birth weight and prematurity, consequent to maternal malnutrition and infection which are more prevalent, place the infant at a nutritional disadvantage right from the birth. Prolonged breast feeding by poorly nourished mothers (Ghai et al., 1970; Mathur, 1975; Shah and Shah, 1975) and poor sanitary conditions prevailing in their dwellings (Ghai et al., 1971; Ghai,
1975) are considered to be responsible for PEM in the pre-school children.

The biological factors also contribute to the problem. A number of workers (Still, 1972; Griffith and Mitchell, 1938; Bhattacharya and Chaudhury, 1962, a, b; Nelson, 1969; Kamakrishnan et al, 1973; Shah, 1975; Walia, 1975; Udani, 1977) have attributed malnutrition to decreased food intake due to socio-economic factors. In the face of illiteracy, cross-cultural confusion, sub-standard housing and hygiene, faulty techniques of weaning and feed preparation, severe PEM is now occurring at a much earlier age.

The role of diet in the causation of two forms of PEM, i.e. marasmus and Kwashiorkor is still a controversial issue. According to Waterlow (1948) and Kagee (1952), Kwashiorkor is a nutritional disease resulting from decreased intake of proteins whereas marasmus results from inadequate intake of calories. Irowell et al (1954) stated that calorie deficiency was the cause of marasmus while deficiency of both proteins and calories led to the nutritional oedema syndrome.
(Kwashiorkor). However, Jelliffe and Lean (1959) blamed that protein-calorie deficiency, during the first three years of infant's life, was responsible for marasmus.

It was, however, realized that it was not possible to make any rigid demarcation between the two extremes of PEM (Waterlow, 1948; Brock and Autret, 1952; Montgomery, 1962). It was proposed by Montgomery (1962) that marasmus and Kwashiorkor are the two clinical extremes of a clinical syndrome and added a generally overlooked point - infants with uncomplicated marasmus are hungry and those with Kwashiorkor refuse to eat. All forms of protein-energy malnutrition were thought to be caused by a reduction in both protein and calorie intake (Dean, 1962; Garrow et al, 1962; McLaren, 1966) although the ratio may vary widely. In case of pure-semi-starvation in which calories/protein ratio is low, primarily because of oxidation of patients own tissues, the clinical syndrome that develops is marasmus (infantile atrophy). However, when calories are curtailed but fairly adequate and protein restriction is severe, the infant presents a altogether different clinical picture, i.e., of Kwashiorkor.
Both the syndromes can be produced in the experimental animals such as rat, pig and monkey (Follis, 1961; Platt, 1961; Ramalingaswamy et al, 1961). When the limiting factor is calories the syndrome is known as marasmus with the complete absence of body fat, normal hematocrit and normal plasma chemistry (McCance, 1960). If, however, a diet rich of calories but inadequate in proteins is provided there is fatty liver and haematocrit and serum chemistry changes to simulate that in children suffering from Kwashiorkor, i.e., low plasma albumin and high plasma amino acid levels (Whitehead, 1964).

Gopalan (1968, 1975) produced evidence that there are no qualitative and quantitative differences in the diets of children in India and other Asian countries who subsequently develop Kwashiorkor or marasmus. They therefore, proposed that the difference in clinical picture reflects not a difference in their diets but a difference in the capacity of the child to adapt. Gopalan (1968) categorically stated that there was no evidence of high energy food being consumed by those children who developed Kwashiorkor, the calories intake, infact, was low.
The protein intake of underweight children was found to be low (1 g/kg body weight) in a dietary survey conducted by Rao et al (1969). This was just adequate as per FAO/WHO Recommendations (1965) and the dietary pattern of children presenting the signs and symptoms of Kwashiorkor or marasmus did not differ materially; both groups were subsisting on identical protein-calorie deficient diet. In addition, marasmus and Kwashiorkor often co-exist in the same community and family, and may, in fact, be seen in the same child while continuing on the same diet, at different times. This was explained by Gopalan (1968) who introduced the term 'adaptation' in the pathogenesis of the two extreme facets of protein-energy malnutrition. As per his hypothesis inadequate diet acts as a stress. If the undernourished child is capable of metabolic adaptation to this stress the end result is marasmus and failure to adapt results in Kwashiorkor.

During the course of development of nutritional marasmus, starvation results in gradual wasting of muscle and subcutaneous fat and, unless carried to excessive
limits, these can be regarded as normal physiological adaptive processes. These catabolic processes probably protect other vital and essential body functions by providing essential amino acids and other metabolites required for the synthesis of different components important for homeostasis (Alleyne and Young, 1967; Whitehead, 1971; Whitehead and Alleyne, 1971). The serum concentration of albumin and β-lipoprotein are also maintained in marasmus, and probably account for the lack of oedema and fatty liver (Coward and Whitehead, 1972).

During the development of classical kwashiorkor, however, the situation is quite different. There is less need for accelerated tissue catabolism because of different feeding pattern. The diet is usually low in protein but adequate or excess in calories. This leads to profound negative nitrogen balance which is clearly reflected in the distorted amino acid pattern. The decrease in concentration of total body protein, particularly albumin and α- and β-lipoproteins are further indications of contracted amino acid pool. There is consequent defective homeostasis resulting in oedema and fatty liver.
Essentially, the difference between classical Kwashiorkor or marasmus is that the former results from a failure of adaptation, possibly because of inhibitory effects of carbohydrates on tissue catabolic processes—mediated through insulin, whilst marasmus represents effective metabolic adaptation via corticoid hormones—but carried out to pathological limits. The above hypothesis has also been supported by a number of workers (Grimble and Whitehead, 1970; Whitehead, 1971; Whitehead and Alleyne, 1971; Lunn et al, 1973; Rutishauser, 1974; Waterlow and Payne, 1975).

Jaya Kao (1974) further explained the above hypothesis to confirm the view that marasmus and Kwashiorkor are two different expressions of same underfeeding stress of PM. She suggested that in Kwashiorkor, it was the failure of the adrenal cortex to respond appropriately—loss corticoids—leading to less catabolic processes thereby limiting the supply of essential amino acids for the synthesis of albumin and lipoproteins etc. Low levels of plasma cortisol in Kwashiorkor have been reported by a number of workers (Ramachandran et al, 1956;
Castellanas and Arroyave, 1961; Alleyne and Young, 1966; Jaya Rao et al., 1968), though some workers have reported adequate adrenal cortical functions in this syndrome (Leonard and Williams, 1964; Leonard, 1973; Lunn et al., 1973). However, plasma growth hormone levels, are raised in kwashiorkor (Pimstone et al., 1966; Beas et al., 1971; Raghuramulu and Jaya Rao, 1974). Marasmus, on the other hand, is an adapted stage with much higher plasma cortisol levels (Alleyne and Young, 1966; Abbassy et al., 1967; Jaya Rao et al., 1968; Tayal et al., 1977) and normal plasma growth hormone levels (Raghuramulu and Jaya Rao, 1974).

THE PATHOGENESIS OF ANAEMIA IN PEM

Anaemia and pallor have been described as the two main clinical features of PEM (Adams, 1954; Woodruff, 1955; Scrimshaw et al., 1955; Mehta and Gopalan, 1956; Adams and Scragg, 1965). Anaemia is an important manifestation of kwashiorkor (Brock and Autret, 1952; Trowell et al., 1954; Adams, 1954; Gopalan and Ramalingaswamy, 1955; Woodruff, 1961). Woodruff (1961), on the basis of
his studies in Africa, emphasized the difficulty of assessing the role of protein malnutrition per se in the genesis of anaemia in Kwashiorkor, since the human PEM is associated with multiple nutritional deficiencies and is often complicated by intercurrent infections and parasitic infestations. This anaemia responds slowly to high protein therapy alone unless the diet is supplemented with specific hematinics such as iron (Trowell and Simpkins, 1957) and folic acid (Walt, 1959). However, Sandoz et al (1963) reported a good response of the anaemia of PEM to a high protein diet without other hematinics.

**Protein, Erythropoietin and Anaemia of PEM**

Knowledge concerning the substances needed for haematopoiesis was first gained by observing the effects of dietary restrictions. Dr. G.H. Whipple and his associates have demonstrated the importance of proteins in erythropoiesis (Whipple and Robscheit-Robbins, 1940, 1947, 1949; Whipple and Madden, 1944, 1947). Since protein is a major component of haemoglobin, it is
reasonable to suspect that protein deficiency will produce a decrease in haemoglobin synthesis, particularly since the turnover rate of haemoglobin amounts to approximately 1.4 g per day in a child with a blood volume of 1200 ml. This amounts to about 10% of his protein requirement.

The importance of dietary proteins in the synthesis of haemoglobin was recognized as early as 1918 by Whipple and Hooper and by Jencks (1922) in their classical experiments in dynamics of protein metabolism and haemoglobin formation which revealed continual but reduced haemoglobin production during starvation in the standard anaemic dogs. Whipple and Robscheil-Robbins (1923-24) did differentiate between iron and protein as the limiting factors in haemoglobin synthesis. Jencks (1922) demonstrated that the regeneration of blood in rats made anaemic by bleeding could be augmented by adequate dietary protein and inhibited by protein deprivation. This was confirmed by McCay (1928).

Since the classical studies of Whipple and coworkers (Laft et al, 1935; Whipple, 1942), the general belief that haemoglobin regeneration takes precedence
over whole body protein utilization, has impressed on clinical investigators the idea that lack of amino acids or protein per se as a plastic material for haemoglobin synthesis does not occur. Person et al (1937) concluded that the haemoglobin formation was more vital than growth and hence got precedence in protein utilization. Hahn and Whipple (1939) stated that reduced protein intake in dogs leads to economic utilization of tissues proteins for continued production of haemoglobin. Whipple in 1942 found that the standard anaemic dogs kept on basal low protein diet could make 40 to 50 g of haemoglobin per week for several weeks in the absence of infection. Furthermore, if protein was fed to anaemic and hypoproteinememic dogs, haemoglobin formation invariably took precedence over production of plasma protein.

Anaemia secondary to protein deficiency was been shown to be reversible (Orten and Orten, 1943). However, Walt (1959) suggested that increased demands of protein used for tissue growth during repletion from Kwashiorkor are responsible for a slow recovery. As an evidence for this theory, he cited the fact that serum proteins are regenerated before haemoglobin. Along the
same lines of thought several investigators (Lien-Keng and Tumbelaka, 1960; Ghitis et al, 1963 a,b; Adams et al, 1967) suggested that protein-deficiency itself leads to bone marrow hypoplasia, probably because of high protein turnover in the bone marrow.

Dietary deprivation of protein leads to anaemia in a variety of experimental animals, including pigs (Cartwright, 1948), rats (Reissman, 1964 a,b; Ito et al, 1964; Ito and Reissmann, 1966) and monkeys (Ghitis et al, 1963 a,b; Sood et al, 1965). The anaemia appears to result chiefly from impaired erythrocyte production since reduced circulating reticulocytes, reduced iron utilization by the red cell, and erythroid hypoplasia of the marrow are observed. However, there may also be a reduction in the red cell life span because of structural defects in these cells (Delmonte et al, 1964).

Protein deficiency in man leading to anaemia may be inferred from the observations on prisoners of war (Leyton, 1946; Walters, 1947) and patients with Kwashiorkor (Ghitis et al, 1963 a,b; Sandozai et al,
et al, 1963; Adams, 1970). The anaemia in Kwashiorkor is complex since there may be deficiencies of many nutrients besides protein along with infections. Nevertheless, in most instances, the anaemia responded to the administration of protein in the form of cow's milk alone (Sandozai et al, 1963; Adams, 1970).

Metcoff et al (1945) reported a comprehensive series of experiments on erythropoiesis by studying changes in plasma volume and red cell volume following acute protein deprivation in rats. They postulated that there exists a dynamic balance between tissue and circulating proteins which could shift to either direction as the need arose. In general most conclusions favour sustained erythropoiesis at the expense of tissue proteins. Anaemia, as indicated by conventional measures of peripheral haemoglobin and corpuscular concentration, has been observed in rats fed a low protein diet (Orten and Smith, 1937; Person et al, 1937; Benditt et al, 1946). Metcoff et al (1945) and Bethard et al (1958) concluded that protein intake is more essential for maintenance of normal erythropoiesis than is total caloric intake and it was suggested that haemoglobin concentration within
the vascular system is more important than red cell volume in regulating erythropoietic rate.

Gomez et al (1954) measured the plasma and blood volumes in malnourished children and stated that the decreased packed red cell volume and haemoglobin are due to an expanded plasma volume which accompanies total overhydration. They further stated that red cell mass of the malnourished children is the same as that of well-nourished children of the same size. In majority of the cases with FEM, the plasma volume is initially decreased and then rises with adequate protein feeding (Cronje et al, 1961; Adams and Scragg, 1965; Alleyne, 1966). The decrease in haemoglobin concentration frequently observed at the beginning of protein refeeding has been attributed to an expanding plasma volume (Cronje et al, 1961; Adams and Scragg, 1965). Alleyne (1966) stated that with therapy there must be a rise in red cell mass since blood volume increases at a rate similar to that of weight while the packed red cell volume remains constant. Furthermore, he showed a very good correlation between plasma volume (ml/kg) and corrected venous hematocrit.
Previous data from human experimentation, particularly those of Keys et al. (1950) showed not only a slight haemodilution but also an absolute fall in total circulating haemoglobin, as semi-starvation progressed. The exact cause for this drop in haemoglobin is, however, not clear from their experiments. Peden et al. (1957) showed a marked initial increase in plasma volume with normal or elevated red cell mass per kilogram of body weight in Cachectic adults. With protein repletion there was an increase in the red cell mass, which was more than twice the percent gain in body weight. Consequently, the role of increased plasma volume and dilution in anaemia of malnutrition are still not clear.

Most of the studies concerning the effect of protein deficiency on erythron have described a moderate degree of associated anaemia, normocytic normochromic in type (Satoskar et al., 1962 a, b; Adams et al., 1967; Gupta et al., 1970 b) with decreased cellularity of bone marrow (Walt et al., 1962; Kondi et al., 1963; Adams et al., 1967). It was suggested that protein itself leads to bone marrow hypoplasia, possibly
because of a high protein turnover in the marrow. Similar type of blood picture and bone marrow morphology could be induced in animals when fed on protein-deficient diet (Person et al., 1937; Orten and Orten, 1943; Bethard et al., 1958; Ghitis et al., 1963a,b; Sood et al., 1965). Reintroduction of proteins in the diet of these animals led to increased erythroid activity (Orten and Orten, 1943; Ghitis et al., 1963a,b).

The observations of Whipple et al. (1956), Keissmann (1964a,b) and many other workers can be summarized in the following way:

**Anaemia develops in animals whose diet is deficient in protein** (Orten and Smith, 1937; Metcoff et al., 1945; Aschkenary, 1961; Bethard et al., 1958) and markedly depressed reticulocyte count (Aschkenasy, 1961) and radio-iron incorporations (Bethard et al., 1958) have revealed the non-regenerative nature of this anaemia. In the same species, a concomitant contraction of plasma volume tends to mask the decline in red cell mass, and measurements of haemoglobin or corpuscular
concentrations alone may be misleading.

An acceleration of erythropoiesis can be induced by subjecting a protein-deprived animal to bleeding (Whipple, 1956; Orten and Orten, 1943). As a result, considerable amount of haemoglobin is regenerated, although less than in a protein supplemented animal. The necessary amino acids are drawn from the body's protein pool, and these observations on blood protein regeneration during reduced or zero protein intake have played a historically important role in the formulation of the concept of the dynamic protein equilibrium (Whipple, 1956).

The anaemia of protein deficiency is reversed by feeding of a complete protein (Orten and Orten, 1943). During the early phase of realimentation, haemoglobin formation takes precedence over the production of plasma proteins (Hobscheidt-Robbins et al, 1943; Handler and Featherstone, 1943).

The protein-deprived animals thus appear to have a mechanism whereby its protein pool is springly used in the physiologic replacement of red cells, but in the presence of anaemia or after repletion with
protein, red cell synthesis seems to occupy a priority position in the utilization of amino acids. However, little is known about the regulatory mechanism involved.

Protein deprivation in rats results in a rapid depression of iron incorporation but realimentation with protein is followed by a normal iron incorporation. Red cell mass has been shown to decline in a linear fashion during protein starvation indicating a removal of senescent red cells after a life span of 70 days.

Another possibility in relation to the anaemia of protein deficiency may be of decreased production of erythropoietin (Ep). Diminished erythropoietin formation or retardation of protein synthesis in erythroid precursors due to lower substrate concentration may be considered as possible cause of erythropoietic depression. It was proved that protein deficiency does not affect the cytoplasmic protein synthesis in the erythroid precursors directly, and the depression of erythropoiesis is attributed to a diminished formation of erythropoietin (Reissmann, 1964 b). Fried et al (1957) reported that erythropoietin production declines in starved rats and attributed this phenomenon to a decrease in
the rate of oxygen utilization. However, Mckenzie et al (1967) reported decreased plasma levels of erythropoietin; but these were not consequent to increased urinary excretion. Foy and Kondi (1966) proposed the decreased production of erythropoietin as a possible mechanism for anaemia of PEM.

In rats, the anaemia of protein deficiency can be prevented or alleviated by administration of erythropoietin (Ito et al, 1964; Reissmann, 1964 a,b; Ito and Reissman, 1966). This observation suggests that the anaemia of protein deprivation does not result from a fundamental lack of haemoglobin substrates at the normoblast level. Instead, when the organism is confronted with a limited supply of amino acids, the erythropoietin control mechanism is altered. This results in a diversion of amino acids to the synthesis of other proteins, perhaps the ones that are vital and more urgently needed. Their results can also be interpreted as follows: Protein deficiency produces a decrease in active tissue mass which lowers the demand for oxygen transport. Consequently, erythropoietin levels are markedly reduced and erythroid atrophy ensues. That
protein deficiency per se is not a limiting factor is evidenced by the fact that these animals responded to hypoxia and erythropoietin injection in a fashion very similar to that of normally fed animals. Waterlow (1968) and Anagnostou et al (1977) explained the extreme sensitivity of erythropoietin production to protein intake on the basis that protein deprivation limits the amount of amino acids available to various tissues to those derived from protein catabolism. The resultant changes in the availability of certain amino acids cause adaptive changes to occur in various organs, which results in limitations on the rate of synthesis of specific proteins such as erythropoietin.

Aschkenasy and coworkers have published a series of papers (Aschkenasy, 1957, 1961, 1962 a,b,c,1964; 1965; Delmonte et al, 1964) on the anaemia of protein malnutrition. They were able to summerize the results in the following way:

1. Acute protein deficiency produces a drop in erythropoiesis. Simultaneously, it brings about a slight drop in the level of haemoglobin which is accentuated
after a month of protein deficient diets. Thyroxine administration prevents the fall in basal metabolic rate and prevents or markedly delays the development of the haematological picture peculiar of protein deficiency. Cortisone administration has a partial effect, primarily preventing the decrease in basal metabolic rate when administered in high doses, though after 65 days of protein deficient diet and cortisone, the haemoglobin drop is similar or even greater than that observed in protein deficient animals.

2. In chronic protein depletion; erythropoietin responsiveness is decreased while there is still some degree of erythroid activity, suggesting the reinitiation of erythropoietin production.

3. Protein deficiency produces a maturation block at the erythroblast level that results in decreased reticulocytosis. Administration of plasma from normal or protein repleted rats induces reticulocytosis, but no increase in $^{59}$Fe incorporation.
Injection of erythropoietin-rich plasma produces both reticulocytosis and increased $^{59}\text{Fe}$ incorporation.

4. There are structural alterations in the red cells produced during protein depletion since the reticulocyte does not mature normally into adult red cell. Furthermore, red cells have decreased survival when injected into normal rats and have an abnormal behaviour to a series of fragility tests. This last abnormality could be partially prevented by incubation of the red cells from protein depleted rats with cholesterol and phospholipids or serum from the normal rats. It was proposed therefore that not only the red cells are abnormal but the serum lacks a protective factor, which could possibly be a lipid fraction. In this regard, it is interesting to note that severely malnourished children have markedly decreased serum lipids.

5. In the final phases of protein deficiency there is increased haemolysis which could be attributed to both defective red cells and on-protective plasma. Iron absorption in the protein depleted rats is altered in that it does not cross the intestinal mucosa.
The common occurrence of a rapid and marked decrease in haemoglobin levels at the beginning of protein therapy, which cannot be fully explained by a rise in plasma volume, has suggested the possibility of a haemolytic component in the anaemia of PEM. Increased serum bilirubin observed by Waterlow (1948) and Jayasekara et al (1951) pointed to the possibility of either increased red cell destruction or decreased liver function in this syndrome. Marvin and Audu (1964) observed the \textsuperscript{51}Cr red cell survival to be reduced in the malnourished children. Zamar et al (1966) also showed decreased \textsuperscript{51}Cr red cell life span. Most of these investigations were conducted, however, during the initial period when the children were receiving high protein diets, which are known to cause erythroid activity. It has been shown that there is significantly increased erythrocyte osmotic resistance, thermal resistance and autohaemolysis in some cases of protein malnutrition (Mankowsky et al, 1967). Asechkenasy (1967) observed the abnormal fragility in vivo of erythrocytes in protein deprived rats. Friedman et al (1967) observed that subjects with Kwashiorkor not receiving specific haematinics
frequently exhibited reticulocytosis during protein feeding without a rise in mean haemoglobin level.

The above observations stimulated Lanzerkowsky et al (1967) to enquire into the role of haemolysis in anaemia of protein-energy malnutrition. They observed considerably reduced erythrocyte survival in patients with protein malnutrition (Kwashiorkor and marasmus) and proposed that this shortened survival time appears to be due to both corpuscular and extracorpuscular factors. Considerable improvement in the erythrocyte survival took place following realimentation with protein. It was thus considered that protein depletion is the primary factor for this reduced survival since improvement occurred on a protein diet with low iron content and without other hematinics.

It has been demonstrated in the animals maintained on an isoealoric but protein free diet, that the erythroid marrow becomes hypopcellular, the reticulocyte count falls, the plasma iron turnover and red cell uptake of radio-iron are markedly reduced, and the red cell mass gradually declines (Heissmann, 1964a,b;
The decrease in erythropoiesis may be caused either by a marrow abnormality induced by protein lack or by a decreased stimulation of the marrow. These possibilities have been distinguished by ensuring adequate stimulus to the erythroid marrow of protein depleted animals in the form of exogenous erythropoietin (Ito and Reissmann, 1966) or augmenting the endogenous erythropoietin output by testosterone (Aschkenasy, 1963) or cobalt (orton and Orten, 1945).

It has been observed that the erythropoiesis continues despite protein depletion. In other studies, anaemia has been produced to provide an increase in erythropoietin production. Robscheit-Robbins et al (1940) have shown that protein depleted dogs rendered anaemic by bleeding are able to produce haemoglobin at a rate three to four times the basal rate. Similarly phlebotomy has resulted in the increased production of red cells (Aschkenasy, 1963; Stekel and Smith, 1969 a,b). Person et al (1937) showed comparable increase in the rate of haemoglobin regeneration in anaemic, iron-deficient rats given iron but maintained on protein-free...
diet. Heath and Taylor (1956) studied a patient with iron-deficiency anaemia who, when given iron, showed haemoglobin formation at an increased rate despite protein depletion and a negative nitrogen balance. This increased erythropoietic response by the protein-depleted animals, when sufficiently stimulated by erythropoietin, ruled out any erythroid marrow abnormality in protein deficiency. It further indicated that when the protein is required for red cell production, it may be drawn from other body tissues despite the depleted state.

If the erythroid hypoplasia of protein deprivation is not caused by marrow dysfunction, it must represent a decreased erythropoietin production. Consistent with this is the observation that animals subjected to acute protein deprivation are exquisitely sensitive to small amounts of erythropoietin (Adamson et al, 1960). Realimentation of Kwashiorkor patients with protein led to the appearance of measurable amount of erythropoietin in the blood and it was followed by an increase in red cell production (Mckenzie et al, 1967; Finch, 1975). Also, the previously mentioned
phlebotomy studies (Kobscheit-Hobbins et al, 1940; Aschkenasy, 1963; Stekel and Smith, 1969 a,b) clearly indicate that erythroid stimulation occurs in protein depleted animals when there is a sufficient cause. In a child with PEM, it may then be postulated that the erythropoietin response curve is displaced to the left, an equivalent output of erythropoietin occurring at a lower haemoglobin concentration. The optimal size of the red cell mass is maintained by feed back loop hypothesis and Finsh (1975) postulated that the following sequence of events might be operating for anaemia of PEM:

1. A decrease in the overall requirements (related to the decreased physical activity and in severe depletion to a decrease in basal $O_2$ consumption) leading to

2. A decreased erythropoietin stimulation resulting in

3. Decreased erythropoiesis.

All these events are considered as an appropriate adjustment to PEM.
It does appear now that erythropoietin lack is a major (if not the only) factor responsible for the 'lazy marrow' of PEM. The mechanism that turns off erythropoietin production is unclear. The turn off occurs too rapidly in the protein starved, iso-calorically fed animals to justify the assumption that lack of amino acids for erythropoietin formation could be responsible. Finch in 1975 speculated that perhaps there might be an intermediary hormonal mechanism that shuts off erythropoietin production. There might be a change in 2,3-diphosphoglycerate (2,3-DPG) or O\textsubscript{2}-carrying capacity in these children. Drenick et al (1964) observed that starved obese adults do not develop anaemia or reticulocytopenia for at least the first 2 or 3 weeks of the therapeutic programme. Therefore, lack of protein alone does not appear to be the switch that turns off erythropoietin.

Iron and Other Hematinics and Anaemia of PEM

It has not been easy to define the effects of PEM per se on erythropoiesis because in PEM, protein
deficiency is commonly and invariably associated with many other deficiencies. The deficiencies of other essentials of red cell production, the prominence of which depends on the diet of the individual patient, regional food patterns, and environmental factors. These may lead to varying degrees of nuclear and cytoplasmic abnormalities in the red cell production and maturation (Viteri et al., 1968). Most of these 'piggy back' deficiencies find less expression in the protein-deficient subject because of the ceiling imposed on the requirements of many nutrients during the period of arrested growth and suppressed metabolic activities. The removal of this ceiling by protein repletion makes the infant particularly vulnerable to the development of associated deficiencies because of the increased demand for all essential nutrients in all aspects of the anabolic state, and particularly for erythropoiesis.

Iron-deficiency is rare in the untreated patient unless there is pathological loss of blood, e.g., with hookworm infestation. The plasma iron is characteristically low but this usually is the result of a decreased transferrin concentration and does not
represent deficiency of iron and iron-deficient erythropoiesis (Cook et al, 1971). The transferrin saturation and red cell protoporphyrin are more valid measures of iron status. Likewise, body folate, although often decreased, is usually not depleted to the extent of producing megaloblastic erythropoiesis. With protein-repletion, however, iron and folate deficiencies become manifest so frequently that their presence becomes essential for the increased erythropoiesis to have an impact on circulating red cell mass (Walt et al, 1962).

It has been proposed that protein-malnutrition, although depressing the erythropoiesis, thereby considerably reducing the red cell mass and slightly decreasing the haemoglobin concentration, involves the marrow in a reasonable adjustment to the overall effects of protein-energy deprivation. A great number of other deficiency states have been seen to be hidden beneath the reduced metabolic requirements of PEM. During any rapid reparation of protein deficiency, a period of particular vulnerability is expected due to the high requirements of other haemopoietic substances, the supply of which is further reduced because of prior dietary limitations.
The above observations recorded in the causation of anaemia in PEM indicate that protein deficiency is not the sole aetiological factor. Scrimshaw and Behar (1961) wrote that 'The exact role of protein deficiency per se in the production of some variant of anaemia' is still not known. Investigators with few exceptions (Altmann and Murray, 1948; Shahidi et al, 1961) have been reluctant to accept a direct cause and/or effect relationship between the lack of protein and presence of anaemia in human subjects. There is no definite relationship between the severity of anaemia and hypoproteinemia (Kondi et al, 1965; Mukherjee et al, 1969; Gupta et al, 1970 a,b). The response of anaemia to the correction of protein deficiency is incomplete and slow (Trowell et al, 1954; Adams, 1954; Viteri et al, 1964; Gupta et al, 1970 a,b). Ghitis et al (1963 a,b) observed that with subsequent protein feeding, there was increased erythropoiesis and the associated deficiencies became apparent and more marked.

Thus, the aetiology of the anaemia may be multifactorial and influenced by local dietary variations. From the diverse parts of the developing world,

Iron deficiency has been thought to be responsible for some of the anaemias observed in severe PEM. This is because of the existence of low serum iron levels, absence of iron in bone marrow, hypochromic microcytic anaemia, and above all the therapeutic response to iron (Manchanda et al, 1969; Mukherjee et al, 1969). Similar observation have been made by other workers (Patel et al, 1960; Pereira and Baker, 1966; Bose, 1970; Lynch et al, 1970; Massa et al, 1978).

Besides iron evidence for the role of other associated deficiencies as complicating factors for the anaemia of PEM can be found in the literature. The complicating factor of iron-deficiency has been clearly described by Hallgren (1953). Other dietary elements
which have been shown to be involved in causation of anaemia in PEM are lack of folic acid (Satoskar et al, 1962 a,b; Pereira and Baker, 1966; Adams et al, 1967) or folic acid deficiency secondary to iron-deficiency (Vitale et al, 1966), riboflavin, niacin and thiamine (Burch et al, 1957; Russo and Balsamo, 1959; Viteri and Gulino, 1964; Foy et al, 1964) and vitamin E (Dinning et al, 1954; Dinning and Day, 1957).

Folic acid deficiency in the diet is accepted as a cause of megaloblastic anaemias which are encountered rather frequently in PEM (Walt et al, 1956; Foy and Kondi, 1957; Lien-Keng and Tumbelaka, 1960; MacDougall and Ross, 1960; MacIver and Back, 1960). The well documented low serum folate and megaloblastosis (Mehta and Gopalan, 1956; Sandstead et al, 1965 a,b; Majaj, 1966; Pereira and Baker, 1966; Halstead et al, 1969; Kamel, 1972) which respond to folic acid in variable manner in adults with PEM, indicate that under special circumstances folic acid deficiency can contribute to the anaemia of Kwashiorkor (MacIver and Back, 1960; Velez et al, 1963; Ghitis et al, 1967).
Chanarin et al (1965) while studying the pregnant women, noted that bone marrow megaloblastosis decreased, when the subjects received iron supplementation. Velez et al (1966) described megaloblastosis in iron-deficient adults, which responded to iron administration. However, Vitale et al (1965, 1966) demonstrated decreased activity of glutamate formimino-transferase in iron deficient rats. This may partially explain some of the megaloblastosis not related to folate and/or vitamin B₁₂ deficiencies in PEM.

Erythroid hypoplasia is commonly seen in malnourished children on admission. Riboflavin deficiency has been held responsible in the development of hypoplastic and aplastic anaemia occurring during the treatment of marasmus and kwashiorkor (Kondi and Foy, 1964). Foy et al (1961) have reported the evidence that hypoplastic or aplastic bone marrow developing in children with PEM following recovery, can be corrected by oral or intramuscular riboflavin administration. Foy et al (1961) suggested that along with protein deficiency, riboflavin deficiency could also be responsible for the erythroid aplastic crisis in kwashiorkor. The fact that
these crisis responded not only to riboflavin administration but to prednisone, made them believe that decreased corticoid activity in PEM could also be invoked as a cause of anaemia.

A role of vitamin E in haematopoiesis of the infants with PEM and megaloblastic marrow have been proposed (Majaj et al, 1963; Dorby, 1968) but has not been established (Baker et al, 1968; Panos et al, 1968). The relationship between vitamin E deficiency and anaemia has been under close scrutiny. However, vitamin E has been shown to play a role in premature infants (Oski and Barness, 1968; Ritchie et al, 1969). Thus vitamin E deficiency is likely to impair the erythropoiesis in humans as it does in non human primates (Porter et al, 1962; Pitch, 1968) and in swine (Lynch et al, 1977).

Vitamin E as a cause of haemolytic anaemia in premature infants, accompanied by reticulocytosis, falling haemoglobin, abnormal erythrocytes and bone marrow findings (William et al, 1975; Gross, 1976;
Bell and Filer, 1981), similar to the ones described in vitamin E deficient monkeys (Osaki and Barness, 1967) is well recognised. Drake and Fitch (1980) reviewed the literature regarding the status of vitamin E as an erythropoietic factor and proposed that it should now be viewed as a potential erythropoietic factor for humans, particularly in patients with anaemia of obscure aetiology such as in PEM.

It has been reported that a good haematological response has been obtained with vitamin E in a group of patients in which marasmus and kwashiorkor were associated with megaloblastic anaemia (Majaj et al, 1963; Majaj, 1966). A number of subsequent studies have supported the relationship between vitamin E and anaemia in PEM. This relationship, however, has been opposed by Baker et al (1968) and Halstead et al (1969) who have found no haemoglobin synthesis in response to vitamin E. In some cases of megaloblastosis, reticulo-cytosis occurs without haemoglobin formation suggesting a non-specific effect of vitamin E on the bone marrow. In a number of cases of anaemia a non-satisfactory
The reticulocyte response to vitamin B and megaloblastic bone marrow changes further suggest that other deficiencies such as that of folic acid may be present along with vitamin B deficiency (Silber and Goldstein, 1970; Kurdoglu, 1971). It was concluded that vitamin B does not appear to play a role in enhancing haemopoiesis in patient with PEM (Baker et al, 1968; Kulapongs, 1975), the main factors responsible for this being protein and iron.

Infection and the Anaemia of PEM

The FAO/WHO expert committee on Nutrition (1971) concluded unequivocally that even a mild degree of PEM in the pre-school child increases the susceptibility to diarrhoeal, respiratory and other serious infections of childhood. There is evidence that protein and energy deficiencies inhibit both types of immunity, thus rendering the child with PEM more susceptible to serious infections (Awdeh et al., 1972; Ramalingaswamy, 1975). Severely malnourished children and even children who are not severely malnourished but who come from an environment in which malnutrition flourishes, often
have a markedly thin intestinal mucosa, similar to that found in intestinal malabsorption. Intestinal flora that are normally found in the lower intestine are often found in large numbers in the upper gastrointestinal tract in these children (Behar, 1975). Changes in the structure and function of endocrine glands are commonly found in severe PEM (Whitehead and Alleyne, 1971). All these alterations are likely to lower the resistance to infections in such children.

Repeated infections tend, in a nutritionally precarious situation as in PEM, to worsen the nutritional status of a young child (Puffer and Seraino, 1973). The mechanisms by which this happens are many and include reduced food intake caused by both loss of appetite and by withholding of nutritious foods and increased nutrient requirements by the disease process themselves.

Infection has been incriminated as a cause of anaemia in severe PEM, since majority of the children suffer from repeated episodes of a variety of infections. Infections cause anaemia by increasing the requirements of nutrients and further limiting the
fulfilment of the bone marrow requirements (Walt, 1959). Erythroid hypoplasia due to infection is still another factor contributing to the anaemia in patients with Kwashiorkor (Kondi et al, 1963). Adams and Scragg (1965) suggested that some of their findings in the anaemia of Kwashiorkor could be explained by infection. However, this possibility was discarded, since the haematological values observed in the two groups of patients with and without infections are not different (Adams et al, 1967).

Acute and chronic infections may also affect the iron metabolism adversely. The obvious effect is the production of anaemia. Infection per se can lead to increased hemolysis and/or defective erythropoiesis. Another mechanism, i.e., iron absorption, whereby infection can affect iron metabolism has been elucidated by Beresford et al (1971). There is a marked reduction in the absorption of iron in children during febrile illness. Bloch et al (1972) reported heavy hookworm infestation in more than 25 per cent of the malnourished children studied by them and observed that it led to severe iron-deficiency anaemia.
It has been suggested that a failure to release iron for the erythropoiesis is an important factor in the pathogenesis of anaemia due to chronic disease (O'Shea et al, 1973). Kuhns et al (1950) observed that in the presence of infection, intravenously administered radio-active iron is mainly taken up by liver and spleen and the amount required for heme synthesis is greatly reduced. It is because of this non-availability of iron for heme synthesis that anaemia of infection may be microcytic hypochromic (Cartwright and Wintrobe, 1952). Malaria and hookworm infestation cause anaemia by haemolysis and haemorrhage respectively.

The similarities observed in the peripheral blood and in red cell survival studies of malnourished children and with those described in chronic liver disease suggested that liver malfunctioning could be responsible for the anaemia (Woodruff, 1955; Lanskowsky et al, 1957). Lien-Keng et al in 1957 also suggested that reduced bone marrow function in severe PEM is due to severe liver malfunction.
Malabsorption and Anaemia of PEM

The marked atrophy of the mucosa of the small intestine and the exocrine pancreas, the abnormalities at subcellular level, the depression of enzyme activities and the reported decreased concentration of conjugated bile salts in the upper jejunum - all conspire to reduce absorption in PEM. Malabsorption is a well recognized feature of infantile malnutrition (Viteri et al, 1964). Nutritional deficiencies result in alterations in the digestive system, which in turn lead to malabsorption and diarrhoea. It is often difficult to determine as to what degree of gastrointestinal alterations are the consequence of/ or the cause of nutritional deficiencies. The various biochemical and pathological alterations of the gastrointestinal tract responsible for malabsorption in PEM have been discussed in recent studies (Gurson, 1972; Viteri and Schneider, 1974; Reddy, 1975b; Alleyne et al, 1977).

The co-existence of diarrhoea and severe PEM was noted since the earliest description of what is
now recognised as protein-calorie deficiency with oedema (Trowell et al., 1954). Later studies have confirmed the presence of diarrhoea in kwashiorkor and marasmus (Scribshaw et al., 1955; Gopalan, 1956; Dean, 1957; Trowell, 1958; Bowie et al., 1963; Truswell et al., 1963; Bowie et al., 1965, 1967; Wittman and Hansen, 1965; Bhattasharya, 1967; James, 1968; Tondon et al., 1968; Wharton et al., 1968; Sawhney and Kaul, 1971). Conversely a large percentage of infants and children attending the hospitals with diarrhoea show evidence of malnutrition (Robertson et al., 1960; Bowie, 1960; Kahn, 1961; Truswell et al., 1963). Some consider that diarrhoea is due to GIT infections whereas others relate it to the pathologic changes of malnutrition (Burgess, 1961).

In kwashiorkor the mucosa of the small intestine undergoes atrophic changes; the normal finger-shaped villi become broad/leaf-like or spade-like which coalesce into ridges and convolutions (Trowell et al., 1954; Burman, 1965; Standfield et al., 1965). According to some workers the alterations in intestinal mucosa in marasmus are still more marked, the villi are almost
absent and the surface epithelium, though of columnar cells, has a marked inflammatory infiltration in the lamina porpria (Mazia, 1961; Lipkin et al, 1963; Barbezat et al, 1967). The main effect of these changes is the decrease in the absorptive surface. However, only mild abnormalities in the intestinal mucosa of most of the marasmic children were noted by Brunser et al (1966, 1968) and Barkel et al (1970). A study conducted in this department by Mehta et al (1982) revealed moderate degree of morphological alterations in the jejunal mucosa of the marasmic children. The malabsorption of all the three major dietary constituents, i.e., fats, proteins and carbohydrates has been attributed to the mucosal changes in PEM. Mucosal abnormalities may also impair the dietary absorption of haematopoietic substances.

**CLINICAL DESCRIPTION OF THE ANAEMIA OF PEM**

**Peripheral Blood Changes**

A moderate degree of anaemia is usual in
uncomplicated cases of PEM. When judged by the standards established in developed countries of the West, a majority of the Indian children will have to be considered anaemic. What level of haemoglobin constitutes anaemia in a given area is in itself controversial. However, employing the criteria laid down by the WHO Expert Group for Anaemias (1959), 61.3 per cent of the severely malnourished children in the hospital were found to be definitely anaemic and their haemoglobin levels were less than 10 g/dl (Mukherjee et al, 1969). Haemoglobin levels less than 10 g/dl have also been reported by a number of workers (Patel et al, 1958,1965; Autret and Behar,1954; MacDougall and Ross, 1960; Kondi et al, 1963; Woodruff, 1961; Patwardhan et al, 1975; Reddy and Srikantia,1970; Verjee et al, 1975; Aggarwal et al, 1980).

In majority of the children with PEM the packed red cell volume corresponds to the level of haemoglobin. Therefore, this anaemia is considered normocytic normochromic. However, the peripheral smear very often shows aniso- and poikilocytosis with occasional target cells, normal white cells, and platelets.
(Trowell, 1937; Altmann and Murray, 1948; Trowell et al, 1952; Adams, 1954; DeMaeyer and Vanderborght, 1954; Woodruff, 1955; Scrimshaw et al, 1955; Mehta and Gopalan, 1956; Lien-Keng and Tumbelaka, 1960; Kondi et al, 1963; Sandozai et al, 1963; Adams and Scragg, 1965; Chopra et al, 1965; Sood et al, 1965; Pereira and Baker, 1966). These studies indicate that a diet low in protein but adequate in all other hematinics, causes only normocytic normochromic anaemia. However, the reported therapeutic response of the anaemia of PEM to haematinics other than protein, change morphologic type, i.e., peripheral smear may be variable depending upon the extent of deficiency of a particular haematinics (Agarwal, et al, 1980).

Woodruff (1955) stated that the cells were large in diameter and thin as in hepatic disease. Similar alterations in red cell size have been described by Lien-Keng and Tumbelaka (1960) and by Viteri et al (1964). Shahidi et al (1961) described two cases with cystic fibrosis and protein deficiency secondary to malabsorption and poor utilization of dietary protein,
who had the type of anaemia essentially seen in uncomplicated cases of PEM in developing areas.

The degree of hypochromia in severe PEM seems to be related to iron-deficiency, associated in the majority of tropical developing countries with hookworm and other blood-tinging conditions (Lehmann, 1949; Trowell, 1948-1949; Prasad et al, 1963 a,b; Bosch et al, 1965). There are, however, several authors who describe microcytic changes with hypochromia in the absence of chronic blood loss (Mehta and Gopalan, 1956; Trowell and Simpkiss, 1957; Lien-Keng and Tumbleka, 1960; Cronje et al, 1961; Kondi et al, 1963; Adams and Scragg, 1965; Patel et al, 1965; Sandstead et al, 1965 a,b; Pereira and Baker, 1966; Mamchanda et al, 1969; Mukherjee et al, 1969; Lynch et al, 1970; Massa et al, 1978).

Macrocytosis has been noted in varying degrees of severity and the prevalence depending on geographic location and deficiency of vitamin $B_{12}$ and/or folic acid (Vander Sar, 1951; Adams, 1954; Woodruff, 1955; Mehta and Gopalan, 1956; Walt et al, 1956; Moller and Back, 1960; Lien-Keng and Tumelaka, 1960; Adams and Scragg, 1962; Satoskar et al, 1962 a,b; Velez et al,
Abnormalities appear in the peripheral blood (morphology, erythrocyte count, Hb*, P.C.V., Red cell indices and reticulocyte count) only after depletion of a particular haematinic store and inadequate dietary intake. Developmental changes in the erythrocyte in the bone marrow complicate the interpretation of peripheral blood smear for a type of anaemia.

Bone Marrow Changes

The bone marrow is not only the source of cells of erythroid, myeloid and megakaryocytic series, but even macrophages and lymphocytes, both of the B and T cell types, are generated in the marrow. Atrophy of the lymphoid tissue, particularly the T-dependent areas and the bone marrow is a feature of malnutrition (Mugerwa, 1971; Smythe et al, 1971; Sood et al, 1965). All the cellular elements in the marrow are affected.

Very little information is available on the
proliferation kinetics of different classes of marrow cells. $^{59}\text{Fe}$ uptake by the marrow, and utilization of the isotope by erythrocytes are both reduced (Bethard et al., 1958; Sood et al., 1965), indicating a depression in the proliferation of erythroid elements. Decreased cellularity of the bone marrow in PEM has been observed by a number of investigators (Walt et al., 1962; Ghitis et al., 1963a, b; Kondi et al., 1963; Adams et al., 1967; Viteri et al., 1968). The stem cell number appears to be also reduced in rats (Deo and Ramalingaswamy, 1965). Bell et al. (1976) have measured stem cell population dynamics in the spleen and bone marrow in mice on different dietary regimens. The capacity of the transfused cells to form haemopoietic colonies (CFU) in the spleens of the recipient X-irradiated normal mice was assessed. A study was also made of the colony-forming cells (CFC) using in vitro technique of Metcalf (1969). The data suggest that both the CFU and CFC are reduced in malnutrition.

The bone marrow has been studied by several investigators, who find a predominant normal maturation
with a reduced erythroid to myeloid ratio (Lambrecht and Holemans, 1955; Adams, 1954; Lien-Keng et al., 1957; Lahey et al., 1958; Line-Keng and Tumbelaka, 1960; MacDougall and Rosa, 1960; Foy et al., 1961; Adams and Scragg, 1962; Satoskar et al., 1962; Ghitis et al., 1963a; Kondi et al., 1963; Sandozai et al., 1963; Adams and Scragg, 1965; Sandstead et al., 1965; Whitaker et al., 1967). Severe erythroblastopenia has been described in malmnourished children not only on admission to the paediatric wards, but also during treatment, usually 2-3 weeks after therapy in begun (Foy et al., 1961; Ghitis et al., 1963a; Kondi et al., 1963; Leame and Simson, 1964). A centripetal reduction of red marrow has been noted in severe PEM at Institute of Nutrition of Central America and Panama (INCAP). Several descriptions of increased erythroid activity appear in the literature among groups of patients who also had increased erythroid to myeloid ratio. MacIver and Back (1960) and Allen and Whitehead (1965), place special emphasis on erythroid hyperactivity in their patients.

The majority of bone marrow morphology descriptions agree that uncomplicated cases of PEM
have predominantly normal erythroid maturation. However, the frequent appearance of larger cells with asynchronic nuclear-cytoplasmic maturation and suggestive changes of erythroid maturation factors deficiency, as manifested by giant bands and metamyelocytes, and loose chromatin which is not typical of the Ehrlich megaloblasts, is evidenced by many reports (Mehta and Gopalan, 1956; Lien-Keng et al, 1957; MacDougal and Ross, 1960; MacIver and Back, 1960; Adams and Scragg, 1962, 1965; Kondi et al, 1963; Ghitis et al, 1965 a; Neame and Simson, 1964; Viteri et al, 1964; Allen and Whitehead, 1965; Sandstead et al, 1965 a, b; Pereira and Harker, 1966; Adams et al, 1967; Whitaker et al, 1967). Several of these investigators also described megaloblastic changes. Very often, only white cell changes, suggestive of deficiency of erythroid maturation factors are found without corresponding changes in the red cell series as reported in megaloblastic anaemia in infancy by Gmelzer and Rutzky (1953). Some workers have shown bone marrow iron to be normal or sometimes even increased in FBA and they could demonstrate the depletion of the iron stores from the bone marrow on feeding high protein diet (Kondi et al, 1963; Adams and Scragg, 1965; Adams et al, 1967).
BIOCHEMICAL CHANGES IN SEVERE PEM

A universal finding in severe PEM is reduction in total serum proteins which affect primarily the albumin and β-globulins (Dean and Schwartz, 1953; Sood et al, 1965; Patwardhan et al, 1975; Agarwal et al, 1980). This alteration is less marked in marasmus than in kwashiorkor (Lahey et al, 1958; Edozien and Udeozo, 1960; Ward, 1962; Kondi et al, 1963; Viteri et al, 1964; Adams and Scragg, 1965). All the investigators who have determined iron binding globulins in PEM have found them to be low. This is also true of caeruloplasmin levels, which were found to be low in PEM (Lahey et al, 1958; Shahidi et al, 1961). The total plasma amino acid levels are reduced, the levels of essential amino acids being lower than those of non-essential amino acids (Edozien et al, 1960; Ayyoyave et al, 1962; Whitehead, 1964; Singh et al, 1973; Ghisolfi et al, 1978; Worthington et al, 1979).

The other most important haemopoietic factor apart from protein, is iron. In this case, much depends
on the timing of the deficiency. Iron-deficiency is more likely to occur in marasmic infants in the first year of life, particularly if they were also born prematurely. Iron deficiency is a common feature in PEM (Patel et al, 1965; Pereira and Baker, 1966; Manchanda et al, 1969; Bose, 1970; Lynch, 1970; Massa et al, 1978) and it is known to be associated with decrease in plasma iron concentration (Sood et al, 1965; Chattopadhyaya and Banerjee, 1975; Brittenham et al, 1981). The decrease in plasma iron is generally due to a deficiency of total amount of iron present in the body which may be caused by a lack of sufficient intake and/or poor absorption of iron or failure of mobilization of iron from the stores.

Plasma iron is derived mainly from RE cells and its level varies according to the marrow demands. The amount of RE storage iron has little effect because normal plasma iron levels have been found with both reduced (Pirzio-Biroli and Finch, 1960) and greatly elevated amounts of stored iron (Harker et al, 1968). Defective RE release due to liver disease, malignancy,
inflammation (Konijn and Hershko, 1977) or ascorbic acid deficiency (Lipschitz et al, 1971) secondary to PEM will also depress plasma iron levels, as well as transferrin concentration (Lahey et al, 1958). A role for transferrin molecule itself in regulating iron absorption has been proposed (Fletcher and Huenne, 1968).

Decreased haemoglobin synthesis has been clearly documented in PEM by Cronje et al (1961) who noted very low values of free erythrocytic protoporphyrin in acute Kwashiorkor, associated with decreased $^{59}$Fe incorporation in erythrocytes even after 14 days of therapy. The plasma iron clearance rate was found to be same as in recovered cases. From these findings, they came to the conclusion that one of the causes of anaemia in PEM is an abnormality in protoporphyrin synthesis and/or a defect in iron transport.

Sood et al (1965) observed flat plasma iron tolerance curves in the protein deficient monkeys. There are several limitations in the interpretation of these curves, since any point on the curve is a net result of
the rate of iron absorption and its clearance (Josephs, 1958; Hallberg and Solvell, 1960). Sood et al (1965) also observed low plasma iron in protein-deprived monkeys after an oral dose of iron may be due to either a rapid clearance of iron from the plasma or low absorption. The common conditions which bring about rapid clearance from the plasma are infections (Bothwell and Finch, 1962), and increased erythropoiesis (Wetherley-Neim and Hutt, 1960). However, there was no obvious focus of infection in the experimental animals and erythropoiesis showed some evidence of depression.

The uptake of $^{59}$Fe by the marrow is known to be depressed in protein deficient rats (Bettard et al, 1958 a,b). It would appear, therefore, that the flat curves indicate a lowered absorption in protein deficiency (Bothwell et al, 1953; Klavins et al, 1963; Higginson et al, 1963; Sood et al, 1965; Lynch et al, 1970; Beresford et al, 1971; Massa et al, 1976) and $^{59}$Fe absorption studies support this hypothesis. Some of the plausible explanations for iron malabsorption are: decreased erythropoietic activity and total red cell mass secondary to the decreased metabolic needs of a markedly reduced
lean body mass (Bothwell et al., 1958; Reyifarje et al., 1959; Massa et al., 1978) and alterations in the structure and function of the gastrointestinal tract (GIT).

Histological and functional changes in the stomach in the form of gastritis (Smythe, 1958; Wittmann et al., 1967; Gracey et al., 1977; Yadav, 1978), reduced output of acid (Mehta and Gopalan, 1956; Adesola, 1968; Shashidhar et al., 1976; Gracey et al., 1977; Yadav, 1978) and pepsin secretion (Shashidhar et al., 1976; Yadav, 1978) are well documented in PEM. It has been shown that organs with high cell turnover such as the GIT, spleen and growing ends of bones of young animals undergo marked atrophy in protein deficiency probably as a result of decreased cellular proliferation (Deo and Ramalingaswamy, 1965).

Structural and functional changes similar to those seen in PEM, have also been recorded in iron-deficiency (Davidson and Markson, 1955; Bademoch et al., et al., 1957; Gupta, 1971, 1972). Since concomitant iron-deficiency in PEM is common, it is not clear whether the changes are the result of PEM or iron deficiency. However,
Yadav (1978) has shown significantly lower acid output in marasmic children with normal tissue iron and haemoglobin more than 7.5 g/dl, indicating that PEM per se also impairs gastric secretory functions.

In marasmus there is a generalised atrophy of the gastrointestinal tract (Passmore, 1947) and reduced gastric secretions could be due to a decrease in their secretory capacity. It is generally believed that normal gastric acidity is necessary for optimal assimilation of food iron (Barer and Fowler, 1937; Moore et al, 1939). Various workers using radioactive iron demonstrated that when iron is given with a meal, gastric acidity affects its absorption (Goldberg et al, 1963; Cook et al, 1964; Jacobs et al, 1966). Most workers agree that the effect of acidity is marked when iron is given in the ferric form (Chaudhry and Williams, 1959; Goldberg et al, 1963; Jacobs et al, 1964, 1966; Jacobs, 1967). Lynch et al (1970) found iron absorption to be decreased in patients with Kwashiorkor when iron was given alone in the form of ferric chloride but when it was combined with normal gastric juice, the absorption was moderately enhanced. Normal absorption of
ferrous sulphate was observed in the same study. There is evidence that the absorption of iron in haemoglobin form is not affected by factors that influence iron salts (Jacobs et al, 1964).

According to Kirch et al (1947), pepsin promotes the absorption of food iron by releasing the iron bound to proteins. Decreased pepsin secretion has been demonstrated both in marasmus and Kwashiorkor (Shashidhar et al, 1976; Yadav, 1978). However, role of pepsin cannot be evaluated separately from that of acid as it is effective only in the presence of acidic pH.

Another possible reason for malabsorption of iron in PEM relates to the changes that occur in intestinal mucosa of small bowel. It is interesting to note that the histological picture of intestinal mucosa of malnourished children exhibits a marked similarity to that observed in tropical sprue or coeliac disease (Baker et al, 1962; Naiman et al, 1964). Iron malabsorption has been seen in coeliac disease (Webb et al, 1967). Malabsorption of iron may be a part of a
general failure in absorption. On theoretical grounds, it might be anticipated that atrophy of villi would lead to malabsorption not only of inorganic iron but also of haemoglobin iron. Iron deficiency per se has been shown to decrease iron absorption (Crawley, 1952; Gross et al, 1976).

Apart from the factors mentioned above, a variety of other factors are known to affect the availability of iron in cases with PEM. Diets inadequate in proteins lower the uptake of iron by intestinal mucosa (Klavins et al, 1959; 1963). Kroe et al(1963) suggested that this may be due to chelation effect of a number of amino acids in the lumen of the gut since these chelates promotes iron absorption. Finch (1975) postulated that longstanding restrictions in dietary proteins, insufficient to produce overt PEM, may still result in iron deficiency.

Infection is still another factor frequently associated with decreased absorption of iron, and malnourished children are prone to it. Massa et al (1978)
observed flat iron absorption curves in a marasmic child. He did not respond to oral therapeutic iron though the absorption curves became normal with antitubercular treatment. Oral iron absorption has been shown to be decreased in the presence of fever (Beresford et al, 1971).

Apart from the cases where a concurrent infection and hookworm infestation could account for iron deficiency in PEM, iron deficiency has been observed without any obvious cause. Decreased absorption of iron has been suggested as a reason for the above findings (Mehta and Gopalan, 1956; Bosch et al, 1965; Pereira and Baker, 1966; Adams et al, 1967). Foy and Kondi (1956) proposed that decreased absorption and increased cutaneous losses of iron and nitrogen in sweat in tropical areas could be responsible for anaemia in hot climate, particularly in poorly nourished populations. Decreased iron intakes in PEM could also be incriminated. Occult iron deficiency may also be present and this only becomes apparent on treatment, uncovering a hitherto latent iron deficiency.
The plasma proteins are low (Patwardhan et al., 1975; Edozien, 1960; Sood et al., 1965; Chattopadhyaya and Banerjee, 1975) so is the serum iron-binding capacity (Edozien and Udoko, 1960; Sood et al., 1965; Chattopadhyaya and Banerjee, 1975; Patwardhan et al., 1975). Unpublished observations of Neale and Soothil (1958) confirmed that the iron binding capacity was reduced to about 1/3rd the normal level, but showed that transferrin was much more profoundly depressed. Antia et al. (1968) reported markedly reduced levels of transferrin in a series of patients with PBM and the levels correlated with independent grading of the severity of the disease. They also observed that transferrin level was far more depressed than the total serum protein level. The defect was rapidly reversible by high protein diet.

The plasma iron is specifically and tightly but reversibly bound to a transport protein, transferrin which delivers its iron to the cells that require it via special receptors (Aisen and Listowsky, 1980). Most of the transferrin is made in the liver (Morton and Tavill, 1977, 1978). Transferrin is not consumed as it delivers
its iron so its turnover is not affected by the plasma iron turnover; the half life being about 8 days. A primary determinant of transferrin level is the rate of its synthesis (excluding conditions with protein loss such as nephrotic syndrome or enteropathy). The synthetic rate is inversely related to hepatocyte ferritin (Morton and Tavill, 1977, 1978) but is also altered by malnutrition beside other conditions (Bothwell et al, 1979). Low values are found in malnutrition (Adams and Seragg, 1965; McFarlane et al, 1969; El-Shobaki et al, 1972; McFarlane et al, 1972; Reeds and Ladi tan, 1976). Morgan and Peters (1971) and Jeejeebhoy et al (1973) have shown a 50 percent reduction in synthesis of transferrin in fasting and protein-deprived rats. McFarlane et al (1970) observed low synthetic rate of transferrin in children with Kwashiorkor, in whom a rapid increase in transferrin concentration occurs after refeeding an adequate diet.

Concurrence of PEM will prevent a rise of the level due to iron deficiency. Total iron binding capacity (TIBC) corresponds to the total plasma transferrin and unsaturated iron binding capacity (UIBC) to that proportion of which the binding sites are not
already occupied by the plasma iron (Ramsay, 1973; Brittenham et al, 1981). Decrease in TIBC may also be caused by a deficiency of ferritin. Brittenham et al (1981) reported low levels of plasma ferritin, iron, TIBC and transferrin in malnutrition.

Although Bainton and Finch (1964) advocated the use of transferrin saturation to help differentiate iron deficiency from other conditions. Yet, transferrin saturation is subject to deviate in those conditions which affect plasma iron and transferrin levels. Serum iron levels are almost always low, but not as low as transferrin levels in PEM. This situation produces high transferrin saturation in spite by low serum iron (Viteri et al, 1968).

There are conflicting reports with regard to iron deposition in the liver in PEM. Some have described no stainable iron (Gillman et al, 1945; Davies, 1950; Bailey, 1966) but McLaren et al (1968) found a considerable amount iron staining, noticeable in the younger, more marasmic infants, despite a low iron status in the malnourished children. There was no evidence that
the haemosiderin deposits disappeared with treatment. The iron in the liver seemed to be unavailable for use by the body and it was suggested that this might be due to copper deficiency, low levels of copper having been found in the liver biopsy material.

Copper is essential for the biosynthesis of haemoglobin because it enhances the release of iron from duodenal mucosa, the hepatic parenchymal cells, and the reticuloendothelial system (Lee et al, 1968 a,b) and its transfer into plasma from where it would become available for haemoglobin synthesis in the bone marrow. Osaki et al (1966) indicated that copper is active as a plasma copper enzyme, ferroxidase, 'Caeruloplasmin' which mobilizes ferrous iron from the stores and enhances its incorporation as $Fe^{+++}$ into transferrin. Iron is thus made available for transport and for the formation of haem and haemoglobin in the bone marrow. The deficiency of copper (Cordano et al, 1964) and Caeruloplasmin (Lahey et al, 1958; Shahidi et al, 1961) has been recorded in PEM.

Vitamin $B_{12}$ levels in serum show wide variations
in PBM and have been reported to be either normal or elevated (MacDougall and Ross, 1960; Adams and Scragg, 1962; Velez et al, 1963; Viteri et al, 1964; Asfour and Firzli, 1965; Majaj, 1966; Pereira and Baker, 1966; Adams et al, 1967; Mittal et al, 1968; Fondu et al, 1978 a). However, on the basis of haematologic and serologic findings and therapeutic response, deficiency of vitamin B\textsubscript{12} has been suggested (Shnier and Metz, 1959; Jadhav et al, 1962; Baker, 1966; Mittal and Aggarwal, 1969; Singla et al, 1970; Aggarwal et al, 1980). In marasmus, vitamin B\textsubscript{12} levels were found to be within normal limits, though lower as compared to the values observed in Kwashiorkor and the rise during convalescence was related to the high protein diet (MacDougall and Ross, 1960; Majaj et al, 1963).

usually on the lower side of normal range although, depending upon the individual children, these may be low (Zuelzer and Ogden, 1946; Zuelzer and Rutzky, 1953; Velez et al, 1963; Majaj, 1966; Pereira and Baker, 1966; Adams et al, 1967). Mattoth et al (1964) found low whole blood folate levels with depressed folinic acid levels only when total folate levels were extremely depressed.

Allen and Whitehead (1965) described increased urinary excretion of urocanic acid after a histidine load in severe PEM. In these children, formiminoglutamic acid excretion was normal in spite of megaloblastic changes in the bone marrow. Formiminoglutamic acid excretion increased to abnormal levels after initial protein repletion; this disappeared with the administration of folic acid. Both the bone marrow picture and the increased excretion of formiminoglutamic acid failed to recover to normal upon administration of vitamin C and B12 and intramuscular iron. In general, there has been a poor correlation between the megaloblastic changes observed in severe PEM and decreased levels of folic acid.
A megaloblastic marrow picture of varying intensity is seen, together with the characteristic changes of giant metamyelocytes. Omar et al (1973) described a combined iron and folate deficiency of the marrow in which the megaloblasts were smaller than even the normal erythroblasts, with nuclear fragmentation and poor haemoglobin filling, and no stainable iron in the stroma. Folic acid deficiency may again be occult, uncovered only by the increased demand of an active erythropoiesis occurring during recovery. High incidence of folate deficiency could have been consequent to a number of factors such as poor body status (Baker, 1966), poor intake of folate in the diet and defective absorption due to generalized malabsorption. Decreased intestinal absorption of folate has been documented in adults with PEM by Ghitis et al (1967). This could easily occur in malnourished children, since they have a variety of absorptive defects and chronic diarrhoea (Matoth et al, 1964; Viteri et al, 1968).

Vitamin E levels in severe PEM have been described to be low by Trowell et al (1954), Behar
et al (1956), Majaj et al (1963), Marvin and Audu (1964), Asfour and Firzli (1965), Whitaker et al (1967), Baker et al (1968), Darby (1968), Adams (1969) and Fondu et al (1978). Low serum vitamin A levels have also been described by Trowell et al (1954) and Behar et al (1956) in cases, with low serum vitamin E. Arroyave et al (1961) showed an increase in serum vitamin A levels upon administration of protein in the absence of this vitamin when the liver vitamin A levels were high before therapy. Waterlow (1948), Burch et al (1957), Schwartz and Dean (1957), Cravioto (1958), Dean (1960) and Mathew and Dean (1960) demonstrated an initial hypolipemia in PAM which improved with treatment with simultaneous disappearance of fatty changes in the liver.

Increased xanthurenic acid and decreased pyridoxine excretion have been shown by Abbassy et al (1959) and Theron et al (1961) in severely malnourished children, which suggests some degree of pyridoxine deficiency. Serum vitamin C levels are often reduced, although not to scurbutic levels (Behar et al, 1956). The levels of thiamine and riboflavin in serum, as well
as in red and white blood cells, are often reduced (Viteri et al., 1964). In tissues, Behar et al. (1956) and Burch et al. (1957) described low normal levels of riboflavin, niacin and total pyridine nucleotides. Russo and Balsamo (1959), also described riboflavin deficiency.

**PATHOPHYSIOLOGY OF ANAEMIA IN PEM**

The contribution by different factors discussed in causation of anaemia in PEM is still poorly understood. However, several hypothesis have been proposed to explain the anaemia of PEM:

1. Data from animal experiments suggests that it is a form of adaptation to a lowered oxygen requirement, the so-called 'adaptive anaemia' (Viteri et al., 1968; Finch, 1975).

2. Many authors consider that the protein deficiency is only one component of a complex haematological picture in which vitamins and minerals deficiencies

3. Both plasma iron and total iron binding capacity are generally decreased in PEM, thus PEM anaemia might present certain similarities to the anaemia of chronic disorders (Cartwright and Lee, 1971).

4. Many workers (Adams et al, 1967; Lanzkovsky et al, 1967; Woodruff, 1968; Adams, 1969; Fondu et al, 1978 a; Brown et al, 1978) believe that protein deficiency directly affects the erythrocytic survival time and the proliferation and/or the maturation of the erythroblasts. The mechanisms of these abnormalities, however, remain obscure. Reduced red cell survival and decreased osmotic fragility have been observed by these workers.
Fonda et al (1978 a) concluded that (1) the anaemia of PEM is not a simple adaptation to reduced oxygen needs, since the red cell survival is reduced and erythropoietin levels are increased (2) the anaemia of PEM cannot be explained exclusively on the basis of deficiencies in protein; iron or vitamins (3) the anaemia is distinct from the anaemia of chronic disorders, which is caused frequently by infections or parasitosis. Contrary to this syndrome the anaemia of PEM shows a normal or decreased marrow iron supply, an adequate erythropoietin production and normal levels of 2,3 DPG (Douglas and Adamson, 1975) and (4) the deficiency of selenium and vitamin E may play an important role in the pathogenesis of anaemia of PEM. The data obtained in animals have shown that vitamin E or selenium deficiencies can lead to an oxidative hemolysis (Burk, 1976). Since selenium is a component of erythrocyte glutathione peroxidase system, the hypothesis of selenium deficiency and associated membrane abnormalities appear to be more striking in determining the red cell survival and osmotic fragility.
Anaemia has been well documented in PEM (Woodruff, 1968; Adams, 1970). Several investigators have suggested that the anaemia of PEM may be secondary to depressed erythropoiesis and/or shortened red cell survival. The reduction in red-cell volume results from both a decrease in the marrow responsiveness to erythropoietin and a decrease in the mean RBC survival (Fondu et al, 1978 a,b,c). The disturbances in the metabolism of the erythroid cells and in particular the metabolic disturbances responsible for increasing the probability of RBC death could result from a defect in the synthesis of the erythrocyte components and/or from an abnormal plasma environment and/or handling the oxidant stress.

Lanzkowsky et al (1967) and Brown et al (1978) demonstrated decreased red cell survivals and decreased RBC osmotic fragility in patients with PEM. These abnormalities can be attributed to the erythrocyte membrane disturbances and infact accumulation of cholesterol and
phosphatidylcholine in the RBC membrane in malnourished children and subsequently decrease with nutritional repair has been observed (Coward, 1971; Brown et al., 1978; Fondu et al., 1978 c, 1980). The increased molar ratio of cholesterol to phospholipid on admission, when compared to recovery, demonstrates the relative accumulation of cholesterol in RBC membrane. Increase in membrane cholesterol contents are known to correlate with increase in red cell membrane surface area and decrease in osmotic fragility (Brum, 1937).

The decreased osmotic fragility and shortened RBC survival in children with PEM would result in premature sequestering of red cells by the spleen, leading to a systemic population of younger cells with an increased RBC membrane lipids and an increased osmotic resistance (Brin and Danon, 1970). Westerman et al (1963) noted a significantly higher lipid concentration in younger, than in older, RBC's. When released from the bone marrow as reticulocytes, the lipid content is 60 to 70 per cent greater than that in mature red cells.

Reticulocytes are remodelled into mature
cells in the first two or three days after release. During this remodelling process, losses occur in reticulum, water, volume, and membrane lipids (Cooper, 1970). It is known that serum lipids decrease in children with PB (Jandl, 1955) and increase with nutritional repair (Truswell et al, 1966). Brown et al (1978) also observed that the serum lipid fractions decrease as the RBC membrane lipids simultaneously increase.

**PEROXIDATION AND ERYTHROCYTE ANTIOXIDANT DEFENCE SYSTEM**

Phospholipid constituents of biological membranes are subject to oxidative degradation which leads to structural damage and, ultimately, to the disruption of cell integrity. The accumulation of phospholipids (which are rich in polyunsaturated fatty acids) and cholesterol and the fact that the erythrocytes are exposed to high concentration of molecular oxygen, may put the erythrocyte in a disadvantage regarding lipid peroxidation (Nockstein, 1965). In fact, Fondu et al (1980) concluded that lipid peroxidation may be a major cause of the shortened erythrocyte life-span in this syndrome.
Peroxidation of erythrocyte plasma membrane lipids has been shown to lead to haemolysis of the cells (Kahn and Mengel, 1965; O'Malley et al., 1966; Jacob and Lux, 1968; Neikkila et al., 1971; Younkin et al., 1971; Barker et al., 1973; Horn et al., 1974). Clinical anaemia has been reported in monkeys (Dinning et al., 1962), in children with \( \text{FA} \) (Dam and Granados, 1945; Whitaker et al., 1967) and in premature human infants (Hasson et al., 1966; Osaki and Barness, 1967; Ritchie et al., 1969; Gross and Melhorn, 1972; Bell and Filer, 1981; Gross, 1976), all of which are associated with increased erythrocyte fragility and are responsive to vitamin E. However, vitamin E does not participate in the process of antioxidation by itself. It works in conjunction with other nutrient derived antioxidants and is a component of cellular antioxidant defense system (Tappel, 1962, 1965, 1969, 1972; Witting, 1965, 1970; Scott, 1978).

The interaction of vitamin E with the Se-activated enzyme, glutathione peroxidase, and sulfur amino acids together with other nutrients renders the lipid-rich regions of erythrocyte membrane much less susceptible to peroxidative damage. The protection of
Figure 2. Cellular antioxidant defence system.

Glutathione cascade:

1. Glutathione peroxidase
2. Glutathione reductase
3. Glucose-G-6-phosphate dehydrogenase

\[
\begin{align*}
H_2O_2 & \xrightarrow{GSH} GSSG + H_2O \\
& \xrightarrow{NADPH} \text{NADP}^+ + \text{H}^+ \\
\end{align*}
\]
erythrocyte membrane against peroxidation is attributed to the normal functioning of glutathione cascade (fig. 2) and several dietary nutrients are known to modulate the efficiency of this cascade and/or act synergistically to alleviate harmful effects of lipid peroxidation.

The vitamin E, which is lipid soluble and sits within the membrane fraction of the human red cells (Chow, 1975), awaits the production of an incipient free radical, or lipid hydroperoxide. This hydroperoxide can be a chain radical terminated by abstraction of a hydrogen atom from tocopherol to form tocopherol quinone, thereby quenching the chain radical process. Protection of the erythrocyte from peroxidative haemolysis by vitamin E has been well established (Friedman et al., 1958; Wytjens, 1956; Horwitt et al., 1968; Draper and Czallany, 1969).

If, however, the vitamin E is ineffective or deficient in dealing with this free radical, the glutathione cascade (fig. 2) can render the free radical oxidant species into a reduced form which inactivates
it chemically, thereby converting reduced glutathione (GSH) to oxidized glutathione (GSSG). The action of glutathione peroxidase (GSi-Px) in reducing free radical and hydroperoxides is dependent upon the availability of GSH. In order for the cycle to continue, GSH is regenerated by reduction of GSSG by reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) via glutathione reductase (GSSG-R) and by de novo synthesis from glutamic acid, cysteine and glycine. The resultant GSH is now ready to serve as an additional reducing agent for another free radical. The NADPH level in turn is maintained by the reduction of NADP⁺ by hexose monophosphate (HMP) shunt enzymes, glucose-6-phosphate dehydrogenase (6-6-PL) and 6-phosphogluconate dehydrogenase (6-6-GL). 

Dietary selenium has been shown to prevent peroxidative haemolysis in the vitamin B deficiency rat (Bieri and Andrews, 1962; Krishnamurthy and Bieri, 1962; Rotruck et al, 1971, 1972). Rotruck et al (1971, 1972) demonstrated selenium-dependent factor in vitamin-B deficient rat erythrocytes, that protected those cells
from haemolysis in the presence of glucose. This finding led to the important discovery that selenium is an essential constituent of the enzyme glutathione-peroxidase (Flohe et al., 1973; Rotruck et al., 1973; Oh et al., 1974; Awasthi et al., 1975). Glutathione peroxidase utilizes reducing equivalents from (GSH) to destroy hydrogen peroxide and fatty acid hydroperoxides that could otherwise initiate oxidative degradation of cellular components including erythrocyte membrane lipids (Mills and Randall, 1958; Cohen and Hochstein, 1963; Christophersen, 1968; Kosower et al., 1969; Tappel, 1969; Flohe, 1971; Lucy, 1972; Chow et al., 1973; Chow and Tappel, 1974). Thus, peroxidative damage to the erythrocyte plasma membrane is prevented by GSH-Px and this activity is dependent on the level of selenium in the diet (Noguchi et al., 1973; Rotruck et al., 1973; Chow and Tappel, 1974).

The function of vitamin E differs from that of selenium: whereas the selenium-containing enzyme GSH-Px is associated primarily with the aqueous phase of the cytosol and plasma (Noguchi et al., 1973), vitamin E is present within the membrane itself (Krishnamurthy and
FIG. 3. INTERACTION OF NUTRIENTS WITH CELLULAR ANTIOXIDANT DEFENCE SYSTEM.
Bieri, 1962; Luch, 1972). Thus, dietary selenium protects against both erythrocyte haemolysis and haemoglobin oxidation under conditions of oxidative stress, while vitamin E protects only the erythrocyte plasma membrane under those conditions (Rotruck et al, 1972). It is, therefore, clear that vitamin E in conjunction with glutathione cascade, essential amino acid cysteine or methionine as a precursor for GSH, and several other nutritional factors, is essential for the production of an optimal antioxidant arsenal which protects against free radical lipid peroxidation and pathology in vivo. While the major cellular defense system appears to function by scavenging free radicals and reduction of hydroperoxides, dietary proteins, lipids, carbohydrates, minerals and vitamins are all closely interrelated to each other and involved in the overall cellular antioxidant defense (Fig.3).

Although the lesion of PEM is primarily that of protein malnutrition, the etiology of the anaemia may be multifactorial and dependent upon local dietary variations. Among the essential nutrients, vitamin and mineral deficiencies have been shown to accompany PEM.
(Verjee et al, 1975). The efficiency of cellular defense system is expected to be reduced in PEM. There are few isolated reports about some of the components of this system. Erythrocyte GSH-Px has been found to be low (Verjee and Behal, 1976; Fondu et al, 1978 d) and same is true for G-6-PD and GSSG-x(Verjee and Behal, 1976).