REVIEW OF LITERATURE
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Protein Energy Malnutrition

Infantile malnutrition due to protein and calorie deficiency must have been common in large parts of the world for centuries, but attention seems to have been focussed upon it only in the early years of this century. Histologically Marasmus (Greek Marasmos, Wasting) was recognised for hundreds of years as being associated with gastroenteritis, a major contributor to high infant mortality. The term 'Kwashiorkor' (taken from Ga language of Ghana) was given by Cicely William (1963) and she recognised that this was a disease characterized by skin and hair changes, oedema, moon face, fatty liver, hypoalbuminaemia and psychomotor changes. Waterlow (1948) and Jelliffe et al (1954) used the term 'Suger baby' to describe, obviously a similar condition as kwashiorkor found in West Indies, where dermatosis was uncommon though oedema was prominent.

Jelliffe (1959) coined the term 'protein calorie malnutrition (PCM) of early childhood' to include the mild and moderate degrees and all the clinical types of the severe degree of malnutrition.
There was a short lived effort through W.H.O. to introduce the term 'Protein Calorie deficiency diseases' but this was abandoned by the expert group meeting in 1970 in favour of Protein Calorie Malnutrition. To replace the term 'calorie' by 'Joule' as a unit of energy lead to the general use of word 'Protein Energy Malnutrition (PEM)'! To emphasize that this was part of the overall energy crisis of mankind, the term energy protein malnutrition or EPM was used by some to give the needed stress to energy deficit (McLaren, 1973).

Good nutrition is the basic component of health. Protein energy malnutrition is the most wide-spread nutritional disorder amongst pre-school children. It is one of the great offenders of childhood morbidity and mortality in the tropical world. PEM has been a major nutritional problem of most countries, mortality being 20 - 30 times higher in developing countries. In India itself 80 million children are malnourished and out of these, 3 - 4 million are suffering from severe protein energy malnutrition (Shah, 1976).

Ghai (1977) reported that 40% deaths in children could be attributed to malnutrition, even though same was often not listed as the primary cause of death in most of the studies.
Rao (1978) reported 1 - 2% incidence of marasmus and kwashiorkor in pre-school children. As many as 60-70% of the children suffered from mild to moderate degrees of PCM, as observed by the author.

Ghosh (1981) estimated that there were about 100 million pre-school children in India. Out of these 3 - 4 million suffered from severe degrees of malnutrition and probably 1 million of them die every year.

**Classification**

Various classifications of PEM have been given from time to time by various workers. Methods of assessing PCM in the community are: clinical, anthropometric and biochemical.

Gomez and his associates (1956) must be credited for giving the first classification of malnutrition using the actual body weight, expressed as a percentage of standard (expected) weight (Harvard, 50th percentile) for that age, as the basis of nutritional status.

<table>
<thead>
<tr>
<th>Grade of malnutrition</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7/ 90% of expected weight for age.</td>
</tr>
<tr>
<td>Grade I</td>
<td>89 - 75%</td>
</tr>
<tr>
<td>Grade II</td>
<td>74 - 60%</td>
</tr>
<tr>
<td>Grade III</td>
<td>&lt; 60%</td>
</tr>
</tbody>
</table>
Its main drawback was that it assumed all children of certain age to have the same weight, irrespective of their size as measured by height.

Jelliffe (1966) modified Gomez's classification by including all cases with nutritional oedema (irrespective of the weight) in severe degree of malnutrition.

McLaren (1967) introduced a simple scoring system for classifying the severe forms of malnutrition based on all the three methods of assessment viz. clinical, anthropometric and biochemical.

McLaren classification

<table>
<thead>
<tr>
<th>Sign</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema</td>
<td>3</td>
</tr>
<tr>
<td>Dermatosis</td>
<td>2</td>
</tr>
<tr>
<td>Oedema + dermatotis</td>
<td>6</td>
</tr>
<tr>
<td>Hair changes</td>
<td>1</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S. Albumin (gm/100 ml)</th>
<th>Total serum protein (gm/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1.00</td>
<td>(≤ 3.25)</td>
</tr>
<tr>
<td>1.00 - 1.49</td>
<td>(3.25 - 3.99)</td>
</tr>
<tr>
<td>1.50 - 1.99</td>
<td>(4.00 - 4.74)</td>
</tr>
<tr>
<td>2.00 - 2.49</td>
<td>(4.75 - 5.49)</td>
</tr>
<tr>
<td>2.50 - 2.99</td>
<td>(5.50 - 6.24)</td>
</tr>
<tr>
<td>3.00 - 3.49</td>
<td>(6.25 - 6.99)</td>
</tr>
<tr>
<td>3.50 - 3.99</td>
<td>(7.00 - 7.74)</td>
</tr>
<tr>
<td>4.00</td>
<td>(7.75)</td>
</tr>
</tbody>
</table>
Score = sum of points.

A score of 0 - 3 is Marasmus;
4 - 8 is Marasmic kwashiorkor, and
9 - 15 is Kwashiorkor.

Either serum albumin or total serum protein is used for
the sake of deriving a composite score.

Certain other classifications have been designed
which use measurements that are taken by a simple
apparatus. These classifications are based on the concept
of age independent criteria. Such methods are supposed
to be used in the field by unskilled personnel. Among
them, Quacstick (Arnold, 1969) method makes use of height
and mid arm circumference measurements.

The ratio of mid-arm circumference to head
circumference measurement has been shown to be age
independent, at least from 3 - 48 months, and remains
unchanged with the variation in sex (Kanawati and

<table>
<thead>
<tr>
<th>Ratio of mid-arm circumference and head circumference</th>
<th>Status of nutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td>∴ 0.310</td>
<td>Nutritionally healthy</td>
</tr>
<tr>
<td>0.310 - 0.280</td>
<td>Mild PCM</td>
</tr>
<tr>
<td>0.279 - 0.250</td>
<td>Moderate PCM</td>
</tr>
<tr>
<td>≤ 0.250</td>
<td>Severe PCM</td>
</tr>
</tbody>
</table>
However, this method is thought to be less accurate and useful only for screening purpose.

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

**Wellcome Classification**

<table>
<thead>
<tr>
<th>Category</th>
<th>Oedema</th>
<th>Deficit in weight for height</th>
<th>Body weight as % of standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under weight</td>
<td>0</td>
<td>Minimal</td>
<td>80 - 60%</td>
</tr>
<tr>
<td>Nutritionally dwarf</td>
<td>0</td>
<td>Minimal</td>
<td>≤ 60</td>
</tr>
<tr>
<td>Marasmus</td>
<td>0</td>
<td>++</td>
<td>≤ 60</td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>+</td>
<td>++</td>
<td>80 - 60</td>
</tr>
<tr>
<td>Marasmic-kwashiorkor</td>
<td>+</td>
<td>++</td>
<td>60</td>
</tr>
</tbody>
</table>

Standard weight is taken as 50th percentile of the Harvard weight for age.

Weight for height = \[
\frac{\text{Weight of patient}}{\text{Weight for normal subject with the same height}} \times 100
\]

Wellcome Classification was probably the first in which an attempt was made to use weight for height, as well as weight for age ratios. Thus, the classification
delineated a specific entity viz.. Nutritional Dwarfs. However, it had some notable deficiencies. It confused between the type and severity of malnutrition and as a result, kwashiorkor appeared to be less severe than the other two types of malnutrition. Again, gradation of deficit in weight for height was not quantitated.

Nutrition sub-committee of the Indian Academy of Paediatrics (1972) classified PCM into 4 grades using 50th percentile of Harvard Standard as a reference point. Classification of the sub-committee of Indian Academy of Paediatrics (IAP) is usually followed for research purposes. Nutrition sub-committee of Indian Academy of Paediatrics met in 1972 and recommended that the classification of malnutrition should be simple and based on weight loss and severity of symptoms. Since growth norms provided by The Indian Council of Medical Research (ICMR) did not reflect growth pattern of children from various socio-economic groups, 50th percentile of Harvard Standard, which was similar to the 50th percentile of the Indian children from upper socio-economic group, was used as reference standard in grading the nutritional status, in IAP classification.
Classification of I.A.P.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Weight expressed as % of reference standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>71 - 80%</td>
</tr>
<tr>
<td>II</td>
<td>61 - 70%</td>
</tr>
<tr>
<td>III</td>
<td>51 - 60%</td>
</tr>
<tr>
<td>IV</td>
<td>⬇️ 50%</td>
</tr>
</tbody>
</table>

Grades I and II were categorised as under-weight while grades III and IV corresponded to clinical status of marasmus. When nutritional oedema was present, letter K was suffixed to the grade denoting kwashiorkor. I-K and II-K would mean kwashiorkor and grade III-K and IV-K would correspond to marasmic kwashiorkor.

Waterlow and Ruti Shauser (1974) published a classification based on weight and height, thus accounting for past as well as present malnutrition. Waterlow maintained that weight for height was independent of age.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Stunting (Height for age)</th>
<th>Wasting (Weight for height)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>⬇️ 95%</td>
<td>⬇️ 90%</td>
</tr>
<tr>
<td>1</td>
<td>95 - 90%</td>
<td>90 - 80%</td>
</tr>
<tr>
<td>2</td>
<td>89 - 85%</td>
<td>80 - 70%</td>
</tr>
<tr>
<td>3</td>
<td>⬇️ 85%</td>
<td>⬇️ 70%</td>
</tr>
</tbody>
</table>
Definition of PEM

W.H.O. (1973) gave the following definition of PEM: "A range of pathological conditions arising from coincident lack, in varying proportion of Protein and calories, occurring most frequently in infants and young children and commonly associated with infection". The condition concerned could be said to range in severity from mild to moderate degrees of malnutrition. It could, as well, be subclinical and only detected by anthropometric and biochemical tests.

Role of nutrition in immunity:

The relation of diet and host resistance has been mentioned in the ancient puranic scriptures of India. The concept that dietary factor influences health is not new. However, in recent years, it has become clear that nutrition is an important part that determines the biological gradient, the natural course of disease including infection.

Nutrition, immunity and infection are known to be closely linked. Inadequate nutrition can alter the immunocompetence and thus increase susceptibility to infection. Infection in turn can adversely alter nutritional status.
There are mainly two types of immune mechanisms which operate against infection. These are 'humoral' and 'cellular' immune mechanisms. Also, there are other non-specific defence factors such as lysozyme complement and opsonins which play an important role in determining resistance to infection. Phagocytic activity and bactericidal properties of leucocytes constitute the first line of defence. Alteration in one or more of these mechanisms may be expected to increase susceptibility to infection.

**IMMUNOLOGICAL SYSTEM**

Immunological system is the part of host defence. Its primary function is to protect against invasion by infectious agent. The major cost of this protection is allergy, auto-immunity and/or rejection of organ transplant. Following are the major limbs of immunological system: T & B lymphocytes, phagocyte and complement.

**Cell mediated immunity in malnutrition:**

Most consistent change in immuno-competence in PEM is in cell mediated immunity. Vint (1937) reported that malnourished children invariably had evidence of thymic atrophy. There was cellular depletion with involution of thymus dependent areas in the spleen and lymphnode. This morphological alteration was reflected
in reduced activity of serum thymic factor which could be responsible for the depression of immune response.

Trowell et al (1954) studied immunological status in kwashiorkor. Autopsy of children with kwashiorkor revealed shrunken thymus. These authors inferred that malnutrition affected the immune response.

Scrimshaw et al (1968) recognized that there was a synergistic interaction between infection and malnutrition.

Phillips et al (1968) observed that severe infection was common in PEM.

Srikantia (1969) observed that mantoux test was often negative in children suffering from kwashiorkor inspite of the clear evidence of active tubercular infection. This observation led the author to conclude that cell mediated immunity was impaired in severe malnutrition.

Ramalingaswami (1969) stated that PEM exerted profound effect on cellular growth and functions. He observed that any cell and organ in the body was involved although to a variable degree. Organ with higher rate of cell renewal such as gut, bone marrow and lymphoid tissue were affected most. There was depletion of lymphocytes and cellular element of immune system.
Smythe et al (1971) demonstrated profound depression of thymolympathic system and cell mediated immunity in malnutrition.

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

Ramalingaswami (1973) observed that malnutrition and infection singly or in combination contributed significantly to morbidity of infants and children in developing countries.

Bhaskaran et al (1974) studied cell mediated immunity by measuring the number of T lymphocytes and incorporation of 3H thymidine into lymphocytes following stimulation with non-specific mitogen PHA (Phytohemagglutinin) Authors found that cell mediated immune response was significantly depressed in malnutrition. They also observed that this response was unaltered in mild grades of malnutrition.

Reddy (1976) also reported that cell mediated response remained unaltered in mild to moderate degree of PEM. Kumar et al (1978) observed depressed cell mediated immunity (CMI) in children with PEM.

Reddy et al (1978) saw that nutrition, immunity and infection were inter-related. They showed that deficient nutrition could alter the immuno-competence, thus increasing the susceptibility to infection.
Puri et al (1980) reported that various parameters of cellular immunity were significantly depressed in severe PEM.

Chandra (1983) observed in his study that there was delayed cutaneous sensitivity to a battery of recall antigens or other chemical sensitizing agents.

**Humoral immunity in malnutrition:**

Majjar (1969) reported normal levels of immunoglobulins in PEM.

Chandra (1972) has reported slightly increased levels of immunoglobulin in PEM.

Chandra (1975) in his study observed that there was a reduced secretory antibody response to live attenuated measles and polio virus in malnourished children.

Puri et al (1980) observed that humoral immunity was not altered in PEM except in the presence of infection, when there was some increase in IgG level.

Udall (1982) reported that antibody response to those antigen which required the help of T lymphocyte and/or macrophage was compromised in malnutrition.
Phagocytosis in malnutrition:

Chandra (1972) showed in his study that energy production for phagocytosis was impaired but there was no demonstrable defect in phagocytes.

Chandra & Ghai (1976) showed in their study that there was a reduced neutrophil response to pseudomonas polysaccharide in cases of malnutrition.

T and B cell lymphocyte:

Harris et al (1945) showed that lymphocytes were involved in the immunological system. It is now recognised that lymphocytes form an indispensable component of body immune system. Although peripheral blood lymphocytes account for only 0.2% of total lymphoid tissue in human body (Osgood, 1954), yet there is a free and extensive migration of lymphocytes via blood and lymph, to various lymphoid organs, connective tissue and bone marrow. This continuous intermingling of a variety of lymphocytes makes them a perfect vehicle for the transportation and dissemination of viruses, bacteria, other antigens and antibody information throughout the body (Yoffey, 1964).

Study of Clamen et al (1966), Devis et al (1967), Millar and Michel (1968) indicated that at least two populations of lymphocytes were involved in most of the
immune responses and that they differed in their anatomic
distribution. Cells of one population were the
precursors of plasma cells. It appears that precursor
bone marrow cells (prothymocytes) migrate to thymus gland
where they are processed become functionally competent
and are transported into lymphocyte compartment (Moore
and Owen, 1967; Owen and Ritter, 1969; Owen and Raff,
1970; Stutman and Good, 1971). They were present in
bone marrow but not in thymus and they corresponded to
bursa dependent system of chicken. Another population
was dependent on and derived from thymus. These two
populations of cells were named as T cell (thymus
dependent) and B cell (Bursa equivalent) (Rettest et al,
1969).

Graves et al (1973) showed that T cells were
concerned with cell mediated (CMI) and B cell with
humoral immunity.

T lymphocytes play a major role in the immune
response of facultative organisms, e.g. tissue/organ
graft and certain infections with viruses. T cells
accounts for as many as 70% of the peripheral blood
lymphocytes while 20 - 25% are B cells (Lukes et al, 1974).
B lymphocytes mature to become antibody producing plasma
cells and play a role in humoral immunity response
(Rowland, 1975). Lymphocytes circulate 4 to 6 times a day.
Cellular membrane is not simply an elaborately constructed container of cytoplasmic organ. Rather it is a complex, highly mobile structure that functions in recognizing certain extra-cellular materials and in transmitting information generated by their recognition to sub-cellular organells. Cell surface characteristics of a lymphocyte and monocyte determines the way in which foreign material will be recognized and dealt with, through an immune response. Antigen specific receptors of T and B cells are essential to generate specific immune responses and may be even required for the distribution of antigen throughout the host (David et al, 1975).

**T lymphocyte**:

It appears that precursor bone marrow cells (prothymocytes) migrate to the thymus gland, where they are processed, becomes functionally competent and are then exported into peripheral lymphoid compartment (Moore and Owen, 1967; Owen and Ritter, 1969; Owen and Roff, 1970; Stutman and Good, 1971). Moreover, profound changes in cell surface antigens marks the stage of T-cell antigeny (Raff, 1971). Thymus compartment contains early thymocytes (T_{10}^{+} T_{9}^{+}), common thymocytes (T_{10}^{+}, T_{1}^{+}, T_{3}^{+}, T_{4}^{+}, T_{5}^{+}) and mature thymocytes.

T lymphocytes are grouped broadly into modulator cells, effector cells and cells producing lymphokines.
Modulator cells are further divided into two categories; those that initiate (helper cells or inducer cells) and those that tend to terminate (suppressor cells) immune response (Reinherz and Schlossman, 1980).

Peripheral T cell compartment contains mature (stage III) lymphocytes which give rise to peripheral T-cell inducer (IND) and cytotoxic/suppressor (C/S) sub-sets.

Helper inducer T cells facilitate antibody production by plasma cell (B cells) and modulate interactions between lymphocytes and accessory cells through the release of lymphokines. The possible mechanism for subsequent termination of antibody production is the activity of suppressor cells.

There appears to be a sub-population of inducer T cell which is required to induce the functions of T suppressor lymphocytes (Morimoto et al, 1981).

In addition to modulatory lymphocytes there are other T lymphocyte as well, viz. cytotoxic effector cells. Cytotoxic effector cells destroy the target cells or provide negative feed back to inhibit antibody response or down regulate inflammatory response (Paul, W.E., 1980). The other function of T lymphocytes is the secretion of lymphokines. These low molecular weight substances
secreted by activated T lymphocytes affect the function of other cells in the surrounding environment. T cells secrete interferon that stimulates other cells to develop anti-viral activity. Macrophage migration inhibition factor secreted by stimulated T cells causes activation and immobilization of macrophages at the site of inflammatory response (Rocklin et al, 1980). The human immune system therefore consists of discrete sub-sets of T cells that are critical for immune homeostasis. It is the balance between effector and regulatory sub-sets that governs the outcome of antigen triggering. The inducer sub-set, is central for the activation of other T cells; B cells and macrophages, as well as for hematopoietic differentiation. Loss of these sub-sets leads to a variety of immunologic disorders, characterized by auto-immunity or immuno-deficiency.

The concept of opposing activity of the modulator lymphocytes is part of the theory of regulation of immune response (Teurog et al, 1981).

Inter-leukin-2 is a lymphokine that promotes activation and division of other T lymphocytes (Gillis, 1983). There is a reduction of circulating thymus dependent T lymphocytes in PEM (Chandra et al, 1983).
B lymphocytes:

B lymphocytes synthesize and excrete specific antibodies and serve as receptors for antigen. Plasma cells represent the extreme form of B cell differentiation. Earlier studies suggested that B cells could be distinguished from T cells by recognition of finger link cytoplasmic processes which were more numerous on B cells. B cells show single antigenic specificity on their surfaces when exposed to the relevant antigen (processed by a macrophage). Under the influence of signals from antigen specific T lymphocyte, B lymphocytes differentiate into plasma cells, which secrete antibody of the same specificity as originally found in its progenator. Lymphocytes having surface IgM, IgG, IgA, IgE, IgD make up the greatest number of B cells in the peripheral blood of adult. Nearly 15 - 30% of peripheral lymphocytes are identified as B lymphocytes. B lymphocytes can be shown to have cell surface receptors that interact specially with certain components of complement. Sheep red blood cells prepared under appropriate condition (EAC) form rosette with human B cells. Lymphocyte membrane possesses temperature sensitive, mobile cell surface components.

Immunoglobulins which are widely dispersed over the cell surface form a "cap" when cells are exposed, at 37°C temperature, to labelled anti-serums (essential to produce cross linking of the surface immunoglobulins).
"Capping" may be effective in producing conformational changes of cell surface receptors permitting necessary redistribution of these important molecules. Such "capping" may be a part of the process of differentiation of lymphocytes to plasma cells (Graves et al, 1973). Adherence of lymphocyte and macrophage to the indicator system is known as EAC rosette. B cells are commonly identified by immunoglobulin on Sig marker.

Phagocytes:

Third component of immune system comprises of fixed phagocytes of reticulo-endothelial system as well as wandering phagocytes (polymorpho-nuclear or mononuclear myeloid cells). The most primitive activity of phagocyte is ingestion of foreign substance which may be degraded, killed or simply transported away from the threatened tissue. The phagocyte is able to secrete at least 50 products into its environment. Large mononuclear phagocytic cells also possess receptors for FC component of immunoglobulins and modified component of complement. The functional relation of these receptors on phagocytes is more readily recognized than those on B cells. Macrophages do not synthesize immunoglobulins.

Method of detection of macrophages by cytophilic nature of antibodies, measures the binding of unaggregated
immunoglobulins, free of antigen, to macrophages. An 
opsonic property of antibody can be detected by binding 
of antigen-antibody complex to macrophages. Macrophages 
possess receptors for complement C₃b, but it is less 
clear which other component is truly important. The 
macrophage has two critical roles - first is that it 
initiates specific immune response. Second function of 
macrophage is that its products exert a modulatory 
function on inflammatory response. Enzyme inhibitor 
such as plasmin and alpha 2 macroglobulin blocks the 
action of proteolytic enzymes. Macrophages product, 
prostaglandin, has been incriminated in suppression of 
lymphocyte function in vitro (Rice et al, 1979). The 
T lymphocyte must "see" the antigen presented at the 
surface of macrophage that has the same histocompatibility 
antigen (Unanu et al, 1980).

Interleukin-1, a macrophage secretory product 
acts in vitro as a stimulent for the proliferation of 
certain T lymphocytes and production of lymphokines 
(Bendtzenk, 1983).

**Complement**

The complement pathways consist of a series of 
proteins in serum with the following features -

1. Sequential activation of inactive precursor (zymogens).
2. Activation of an increasing number of molecules in subsequent steps of the sequence (Cascade).

3. Amplification of propagation of inflammation by products of activation. There are two pathways of complement system - classic pathway and alternate pathway. The complement system is invariably affected in PEM. The total haemolytic complement activity may be reduced as also the level of C3. Infection may produce acute phase reactants, increase in complement activity, but more often a further depression in the concentration of complement protein, partly as a result of consumption in the antigen-antibody reactions. Due to the change in complement system in PEM, opsonic function of plasma may be reduced.

**BIOCHEMISTRY OF MALNUTRITION**

In 22 years since the classic treatise 'kwashiorkor' appeared (Trowell, Davies and Dean, 1954) there has been a significant advancement in the understanding of the alteration in the biochemistry and physiology of PEM. During this period, emphasis has shifted from the description of biochemical findings to a description of functional change and its control mechanisms. It had to be realized that these changes could not be regarded simply as impaired or disturbed functions, and Waterlow's (1968) concept of an adaptation in the child to an
environment ranging from hostile to suboptimal has been a major contribution to the understanding of nutrition and malnutrition.

1. **Protein and amino acid metabolism**:

   In the child or infant with moderate to severe PEM total body protein/albumin is reduced. Some organs are much more affected. Plasma protein is reduced in kwashiorkor, the greatest reduction occurring in the albumin fraction (Waterlow, Cravioto and Stephen, 1960).

   Muscle fat in marasmus is more severely depleted (Standard, Wills and Waterlow, 1959; Garrow, Fletcher and Halliday, 1965; Halliday, 1966, 1967; Waterlow and Alleyne, 1971).

   In kwashiorkor, albumin fraction was reduced as observed by various workers (Waterlow and Alleyne, 1971; Whitehead and Alleyne, 1972).

   (i) **Albumin** :- It is now well established that the catabolic rate of albumin in severe PEM is reduced to about half the rate found after recovery (Cohen and Hansen, 1962).

   In a study, James and Hay (1968) established that with a low protein diet the rate of synthesis was lower in malnourished children than in the recovered child.
Albumin synthesis rate rose and fell promptly when protein intake was increased or decreased and these changes were more marked in the malnourished than in the recovered child. It was concluded from the studies that a reduction in protein intake was followed rapidly by a decrease in the synthesis rate of albumin and catabolic rate was not directly affected by dietary protein intake nor by the rate of synthesis or plasma concentration of albumin.

In cases of protein energy malnutrition, total serum proteins particularly albumin and transferrin were significantly decreased. This has been attributed to the increased antigenic challenge of chronic infections in malnourished children (Cohen and Hansen, 1962; Alvarado and Luthringer, 1971).

Similar findings as have been reported in other children who suffered from malnutrition (Jose and Welch, 1970). Hypoalbuminemia was more pronounced in malnourished infected children, reflecting their lower nutritional status. This also appeared to be reflected in lower intestinal albumin levels found in this group.

Serum albumin, total serum protein levels repeated two weeks after nutritional therapy showed increased mean serum levels of albumin and total serum proteins (Neuman et al, 1975).
Bell et al (1976) worked on immunoglobulin and albumin levels in PEM from Caucasian, Indonesian and Australian children. They found that there were decreased levels of serum albumin.

(ii) Globulins :- Gitlin et al (1958) in their study, noted a diminished rate of synthesis of albumin but not of gamma globulin in uncomplicated kwashiorkor. In kwashiorkor complicated by infection IgG synthesis was increased three-folds.

Cohen and Hansen (1962) also noted that gamma-globulin metabolism, unlike that of albumin, was unaffected by nutritional status and in the presence of infection the synthesis rate was greatly increased.

(iii) Total body protein turn-over :- The effect of diet on protein turn-over had been studied in rats and in malnourished children. Waterlow and Stephen (1967, 1968) in their study on rats concluded that protein turn-over did not reduce by short periods of starvation or protein deprivation but a 30% reduction occurred after 5-6 weeks on low protein diet.

Waterlow (1975) and Young et al (1975) have shown that the rate of overall protein synthesis decreased with age.
(iv) **Protein turnover in different tissue** :- Protein synthesis in the liver was well maintained, while it was greatly reduced in muscle when a low protein diet or amino acid deficient diet was fed to rats for 3 days (Millward, 1970; Millward and Garlick, 1972).

(v) **Amino acid metabolism** :- In severe PEM total plasma amino acid concentration was reduced to one-half the normal value. In kwashiorkor there was a fall in the plasma concentration of most of the essential amino acids. Marked reduction was seen in the case of branched-chain amino acids and threonine, while lysine and phenyl alanine was less affected. The plasma concentration of non-essential amino acids was fairly well maintained or even increased (Arroyave et al, 1962; Holt et al, 1963).

2. **Lipid Metabolism** :

Fatty liver was a striking feature in kwashiorkor and was described by Williams (1933). Chemical determination of liver lipid at post-mortem had shown a severe degree of fatty infiltration of the liver in kwashiorkor even in the absence of hepatomegaly (Waterlow, Bras and Depass, 1957).

**Mechanism of fatty liver** :- Severe fatty infiltration leading to hepatomegaly carries poor prognosis and fat content in the liver of 40% was associated with high mortality (Waterlow, Cravioto and Stephen, 1960).
In fatty liver, the liver size increased, became pale in colour and firmer in consistency. Glycogen levels increased in the liver cells in kwashiorkor (Waterlow & Weisz, 1956; Stuart et al, 1958).

Fat accumulation was less severe or even absent in children in marasmic type of malnutrition (Chowdhuri, Bhattacharya and Basu, 1961). Hepatomegaly due to fatty liver was more constantly associated with fatal outcome in kwashiorkor. In Jamaica a high degree of oedema was also observed (Montgomery, 1963).

Excess of cholesterol in vitro altered the lipid composition of lymphocytes and granulocyte membrane and impaired their function, led to increased susceptibility to certain infections. Large amounts of fatty acid inhibited in-vivo primary and secondary response to certain antigens and in-vivo lymphocyte response to mitogens. Reticulo-endothelial system function was inhibited, granulocyte migration and bactericidal capacity was impaired.

Excess of polyunsaturated fatty acid supressed granulocyte function and delayed hyper-sensitivity reaction. Increased glycogen levels in liver cells were also associated with reduced levels of glucose-6-phosphatase (G6PD) activity (Fletcher, 1966).
Most of the available evidence supports the idea that liver has a reduced ability to dispose triglycerides which therefore accumulates and gives rise to fatty liver. The low fasting level of serum triglycerides in untreated case, the fact that the liver fat was virtually all triglyceride and that marked rise in serum triglyceride accompanied defatting of the liver when treatment begun, was all consistent with this theory. Very low density lipoproteins (d \( \leq 1.063 \)) equivalent to pre-beta fraction, is thought to be responsible for the transport of fat from the liver to plasma (Flores et al, 1970).

Plasma lipoprotein had important immunoregulation affect on cholesterol and high density lipoproteins essential for lymphocyte function. However, excess amount was immuno-suppressive. Very low density lipoprotein specifically inhibited protein and DNA synthesis in many cells and lymphocytes.

There was also a possibility of increased hepatitis antigen in kwashiorkor (Suskind and Olson, 1973). Laboratory animals deprived of fatty acid showed lymphoid atrophy and reduced antibody responses both to T-dependent and T-independent antigen (Newbarne et al, 1981).
3. **Body fluid**

(i) **Total body water** :- A study of J. Patrick, Reeds, Jackson and Ricou (1957 Unpublished) showed that TBW % was the same before and after recovery of PEM. Garrow, Smith and Ward (1968) concluded there was over hydration in both kwashiorkor and marasmus and that an expansion of the extra-cellular fluid space accounted for most part of the increase in total body water.

(ii) **Extracellular water** :- has been estimated in vivo in PEM by measuring the distribution space of thiocynate, thiosulphate (Gollan, 1949; Kerpel-Fronius and Kovach, 1948; Brinkman et al, 1965; McLaren and Pellett, 1970) or bromide (Alleyne, 1967; Graham et al, 1969).

(iii) **Intracellular water** :- was determined indirectly by subtracting extracellular water from the total body water and result obtained were therefore open to some criticism. Intracellular water was lost in patients suffering from kwashiorkor and that during recovery there was an absolute increase in intracellular water, presumably due to shift of water into intracellular compartment (Brinkman and Hansen, 1963).

(iv) **Oedema** :- Hypoproteinaemia predisposes to oedema. Montgomery (1963) demonstrated a significant correlation of hypoproteinaemia with the degree of oedema.
Srikantia (1968) has proposed that an increased activity of anti-diuretic hormone (ADH) played an important role in the formation of oedema in kwashiorkor. He postulated that there was defective inactivation of ADH due to structural and functional changes in the liver, which also promoted release of ferritin into plasma. Srikantia (1968) has reported the presence of ferritin in the plasma in kwashiorkor and in nutritional oedema in adults, but not in the plasma of marasmic infants or normal control.

Increase in aldosterone secretion has also been postulated as a causative factor of oedema in kwashiorkor. But there was no good evidence that increased aldosterone concentration or secretion rate was responsible for the oedema in kwashiorkor (Migeon et al, 1973).

Albumin affected plasma colloidal osmotic pressure. Colloidal pressure did not fall significantly until albumin concentration fell to between 25.1 and 27.5 gm/litre (Coward, 1975). An increase in serum globulin concentration resulting from infection explains that colloidal osmotic pressure was maintained despite an initial fall in plasma albumin concentration.

Halliday (1967) in his data on whole body analysis, did not support the suggestion of Frank et al (1975)
that preservation of subcutaneous fat was necessary for the development of pitting oedema.

4. **Skin and Hair change**:

Skin gets depigmented and in *very severe cases,* is covered with darker shiny patches which then crack like patched earth and has been described by William (1933) as 'crazy pavement'. The cracks between the dark patches remain infected and desquamated followed by ulceration particularly in the folds and crease of axillae, groins and other area.

The hair loses its lusture and natural curls. Colour changes of hair in dark haired races occur towards brown, red, blond and to near white. The changes start at the hair line and could affect separate segments of length of each hair to give the 'flag sign' (Chauarria, 1953). Brittleness, thinness and sparseness of hair was accompanied by easy 'pluckability'. The hair changes, particularly the pigmentation, were probably more related to the duration of malnutrition and could be completely absent in acute protein energy malnutrition.

**T and B cell count**:

T and B lymphocytes can be identified by various methods. Human B cell possess surface immunoglobulins detectable by direct immunofluorescence. They also
possess receptors for aggregated immunoglobulins, for antigen antibody complexes and for third component of complement. It is believed that rosette is formed by rapid release of metabolised receptor substances on the living cell-surface. These receptor substances are possibly positive bivalent ions since ethylene diamine tetracetic acid will block the rosette formation (Jondal, 1972). These receptors are detected by erythrocytes coated with antibody or complement that surrounds B lymphocyte in a cluster (Wybran and Fundenberg, 1973).

Presently, the spontaneous formation of rosette with sheep erythrocyte appears to be a specific property of T lymphocytes and membrane immunoglobulin detectable by immunofluorescence constitutes the most reliable marker of B cells (Saligman, 1974). This method of identifying lumen T cells by directing antibodies against T & B lymphocytes and these rosette formation was described by Fundenberg (1975). However, the fundamental nature of rosettes formation is still not known.

Comparable results using either ethylene diamine tetra acetic acid (EDTA) or heparin (an anti-coagulant) for rosette testing have been reported. Handfield et al (1975) and Fairbank (1976) reported that as the concentration of heparin was increased in the test system, the percentage of T lymphocyte rosette formation decreased. Normally
there are more than 1500 circulating T cells/mm$^3$, each being less than 10 μ in diameter. In some cases of T cell deficiency, number of lymphocyte count is normal or even elevated but the lymphocyte are larger than 10 μ in diameter. Monocytosis and eosinophilia are commonly associated with T cell deficiency (Buckley et al, 1972; Rose and Friedman, 1976).

Steel et al (1974), Chisholm et al (1976) noted that T and B lymphocyte rosette formation is affected by temperature, incubation time and red cell to lymphocyte ratio. The data obtained indicate that the SRBC/lymphocyte ratio is a primary variable in determining the percentage of T cell rosette formation.

Variation of lymphocyte count with age and sex:

Zochorski and co-workers (1971) noted that there was no significant variation of lymphocyte count with age and sex.

Wybran et al (1972) found that there was no difference in T and B cell counts of infant and children. Weeksler and Hutteroth (1974) found no difference in total lymphocytes and relative number of T lymphocytes in the peripheral blood of young children and adult individuals.

In most of the studies regarding the deviation of T lymphocyte and B lymphocyte counts in disease,
age characteristics of the control data have not been
given, though Elhilali and associates (1978) have
emphasized the importance of using age matched control.

**Normal distribution of T and B lymphocytes:**

Fleisher et al (1975) studied the sub-population
of T and B lymphocytes in the peripheral blood of children
and adult using E and EAC rosette assays. Children under
18 months of age were found to have decreased percentage
of E-binding(T) lymphocytes and an increased percentage
of EAC binding (B) lymphocytes as compared to older
children (18 months to 10 years) and adults. The absolute
number of E-binding and EAC binding lymphocytes was
increased in children under 18 months of age.

Neighburger et al (1976) studied the distribution
of T and B lymphocytes in peripheral blood of children
and adult. They found the following distribution.

<table>
<thead>
<tr>
<th></th>
<th>T cell %</th>
<th>B cell %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>44.0 ± 4.2</td>
<td>30.4 ± 3.1</td>
</tr>
<tr>
<td>Adult</td>
<td>46.3 ± 1.8</td>
<td>26.5 ± 2.3</td>
</tr>
<tr>
<td>Age group</td>
<td>T (E-binding cell)</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Range</td>
</tr>
<tr>
<td>&lt; 18 months</td>
<td>50.2 ± 8.7</td>
<td>33 - 67</td>
</tr>
<tr>
<td>18 months - 10 years</td>
<td>56.8 ± 5.9</td>
<td>45 - 69</td>
</tr>
<tr>
<td>Adult</td>
<td>64.0 ± 6.9</td>
<td>51 - 78</td>
</tr>
</tbody>
</table>

**B (EAC binding) cell**

<table>
<thead>
<tr>
<th>Age group</th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18 months</td>
<td>26.2 ± 6.3</td>
<td>14 - 39</td>
<td>1530 ± 540</td>
<td>470-2590</td>
</tr>
<tr>
<td>18 months - 10 years</td>
<td>22.7 ± 3.4</td>
<td>16 - 29</td>
<td>720 ± 280</td>
<td>170-1270</td>
</tr>
<tr>
<td>Adult</td>
<td>17.2 ± 3.9</td>
<td>11 - 23</td>
<td>540 ± 170</td>
<td>170-510</td>
</tr>
</tbody>
</table>

Variation of total average rosetting values and range for adult control and PCM children:

Chandra et al (1972) observed that in PEM T lymphocyte rosetting is decreases while B lymphocyte rosetting value remains unaffected (except in children who acquired infection in whom it is increased 3 folds). Betsy and Bang et al (1975) also supported the finding that T lymphocyte rosetting was decreased in PCM.
Prabha et al (1978) reported reduction of absolute number of T and B lymphocytes in blood, thymus, lymph nodes and spleen in PCM.

<table>
<thead>
<tr>
<th>PCM type</th>
<th>Degree</th>
<th>Average % B</th>
<th>Average % T</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>+++</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td>k</td>
<td>++</td>
<td>29</td>
<td>43</td>
</tr>
<tr>
<td>M</td>
<td>+</td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>NE (Nutritional oedema)</td>
<td>+++</td>
<td>51</td>
<td>53</td>
</tr>
</tbody>
</table>

Average PCM range

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>13-39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23-63</td>
</tr>
</tbody>
</table>

Average adult control

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>22-38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54-72</td>
</tr>
</tbody>
</table>

Averaged B rosetting values in children with PCM, with and without clinical infection at the time of admission

Average B cells

(36%)  
(Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfected) 26%  (Uninfecte
Variation of serum albumin in infants and children with PCM alongside control values:

<table>
<thead>
<tr>
<th>Protein</th>
<th>Normal value (gm/100 ml.)</th>
<th>Severely malnourished</th>
<th>Moderately malnourished</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Kwashi-orkor</td>
<td>Marasmus</td>
</tr>
<tr>
<td>Albumin</td>
<td>72.5</td>
<td>2.4</td>
<td>2.0</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Ferghsson and co-workers (1974) observed that children with low T cell percentage had severe hypoalbuminemia (≤2 gm/100 ml) as well.

Change in Hb% and lymphocyte count in PCM and control:

<table>
<thead>
<tr>
<th>Normal value</th>
<th>Severely malnourished</th>
<th>Moderately malnourished</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb 7.0 gm/100 ml.</td>
<td>60.0%</td>
<td>17.5%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Lymphocyte count 600 - 4,500</td>
<td>2860 ± 300 (9%)</td>
<td>3380 ± 240 (5%)</td>
<td>3270 ± 290 (2%)</td>
</tr>
</tbody>
</table>

All these changes were reported by Neumann et al (1975) in their studies.
Delayed hypersensitivity in Protein Energy Malnutrition:

In vitro T-lymphocyte function can be measured by delayed hypersensitivity reaction using a variety of antigens to which majority of older children and adults have been sensitized. Generally, useful skin test antigens are 1:100 dilution of tetanus toxoid, protein purified derivative (PPD), histoplasmin, mumps virus, extract of candida, Trichophyton, phytohaemagglutinin (PHA) and Dinitrochlorobenzene (DNCB).

Epstein and Kligman (1959) reported that a single application of DNCB sensitized over 90% of a large series of normal control, the ability to initiate and manifest DNCB hypersensitivity was not predictive of any form of morbidity in either age group.

Aisenberg (1962) in his study of DNCB test using 1000 ug/0.1 ml concentration of DNCB for sensitizing dose and 50 ug/0.1 ml for challenge dose reaction was read day 2, observed severe itching present at the site and also vesiculation, which was less common. He opined that both indicated sensitization. Reaction was graded as follows:

3+ spontaneous flare occurring at both sensitizing and challenge dose sites.

2+ spontaneous flare occurring only at sensitizing dose sites.
1+ absence of spontaneous flare but reapplication of challenge dose causing an equivocal delayed hypersensitivity reaction.

-ve No reaction. No spontaneous flare occurring even after reapplication of challenge dose.

Depression of CMI may partly determine the pattern of infection in PCM. Various postulates have been put forward by Scrimshaw et al (1959) with emphasis on the effect of specific nutritional deficiency on various virus and bacterial growth. In some cases the germinal centres were so depleted that depression of humoral immunity might also have been expected.

Poor reaction denote depression of the CMI. Depression of CMI has been postulated in PCM to explain negative DNCB reaction (Harland, 1965).

Depression of CMI in PCM could be the result of an absolute or relative deficiency of amino-acid for cell multiplication. Alternatively, children with PCM are known to have raised level of plasma cortisol (Rao et al, 1966; Alleyne, 1966; Rao et al, 1968), which can depress the thymo-lymphatic system.

Smith et al (1971) reported that in the PCM group, 12 (70%) had no reaction to DNCB and 5 (30%) had a grade I reaction, whereas in the control group, none failed to react.
6 (32%) had grade I, and 13 (68%) grade II (++) reaction. The response to chemical substances was significantly less in PCM group ($P \leq 0.01$). A significantly greater loss of germinal center was found in all cases with PCM, with the greatest loss in kwashiorkor group.

Malnourished children have impaired immunocompetence (Smythe et al, 1971; Chandra, 1972; Seth and Chandra, 1972) and cell mediated immunity is consistently depressed.

T cell undergo mitosis in response to stimulation in vitro by specific antigen or by mitogen (plant derived material) that perturbs the lymphocyte membrane and trigger's cell division (Smythe et al, 1971). Skin testing is an important test for the assessment of cell mediated immunity. Catalone et al (1972) developed a method for DNCB contact sensitization which response to antigen is evaluated by an experienced observer by noting induration, erythma, oedema, as well as size of reaction. Positive test has a value in establishing the presence of normal T cell function but negative skin test is considered as inconclusive evidence of deficient T cell function, particularly in young children with limited antigen experience. Direct sensitization with 2, 4 dinitrochlorobenzene can be performed followed by a subsequent challenge of cutaneous reactivity with the same material
The capacity to become sensitized to a new antigen may be tested by application of DNCB to the skin, followed at 2 week later by patch testing at different sites with the same material. Thus ability of an individual to develop cell mediated immunity denote can be determined by applying directly to the skin, a chemical DNCB to which individual had not been previously exposed. The chemical combines with skin protein to form an immunogenic substance that stimulates sensitization of T cell to DNCB. The reapplication of DNCB on skin if CMI is intact. DNCB skin test for the assessment of cellular immunity has advantage over other intradermal tests. Reliance upon previous exposure to allergen is unnecessary and circulating antibody does not develop with contact sensitization which renders it more exact test for testing cellular immunity.

Schlesinger and Stekel (1974) carried out DNCB skin testing in malnourished, healthy and infected patients. They observed that skin test response was depressed in malnourished children in comparison to healthy control. But, infected patients as compared to well nourished infant showed intense positive reaction with DNCB.

Bang et al (1975) confirmed that DNCB was generally to be considered as a measure of cell mediated immune response. He also observed that kwashiorkor had
lower T lymphocyte rosetting values than in case of other types of PCM. Children with kwashiorkor seemed to have more disabilities.

Watts (1980) postulated that in malnourished children, atrophied thymus was the most likely cause for impaired cell mediated immune response. Author further observed that DNCB test could also be used in children before subjecting them to immunization procedure, when a sensitizing dose was applied, the immediate reaction produced expressed a general inflammatory response. Sensitizing dose was expressed by the occurrence of a spontaneous flare 7 to 14 days later, or by reaction to a challenge dose of DNCB. Equivocal reactions required histologic examination.

Study of Sanjeev et al (1981) revealed that malnourished children developed impaired reaction to DNCB (45.9%). They compared DNCB reaction in control and malnourished children and reported his observation as follows:

<table>
<thead>
<tr>
<th>DNCB reaction</th>
<th>Control group</th>
<th>Study group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>+ 3</td>
<td>11</td>
<td>36.7</td>
</tr>
<tr>
<td>+ 2</td>
<td>12</td>
<td>40.0</td>
</tr>
<tr>
<td>+ 1</td>
<td>3</td>
<td>10.0</td>
</tr>
<tr>
<td>-ve</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
</tr>
</tbody>
</table>
As previous study about relation of DNCB reactivity with age, Koster et al (1981) in his study observed that the ability to initiate and manifest DNCB hyper-sensitivity was correlated with weight for age in 12 - 35 months old group with weight for height in the 7 - 36 months age group. Since prolonged episodes of diarrhoea will result into the failure of immune defenses, the frequency of episode of diarrhoea lasting 7 - 14 days was compared between anergic and not anergic children. Among children 7 - 36 months of age, anergic children were more likely than not to have diarrhoeal episode lasting 7 14 days. The percentage of diarrhoeal episodes lasting 7 14 days was higher in anergic than not anergic children.

Elber et al (1981) reported malignant melanoma cases to be less reactive than normal individuals in skin testing to DNCB. Possibility of DNCB negative reactivity increase as the disease progressed.