DISCUSSION
DISCUSSION

HAEMATOLOGICAL CHARACTERISTICS AND IRON METABOLISM IN ANAEMIA OF PEM

In the children suffering from protein-energy malnutrition (PEM) of the present series, anaemia was obvious as revealed by reduced haemoglobin levels (Fig. 4), packed cell volume (Fig. 5) and red blood cell count (Fig. 6). The red cell indices, i.e., mean corpuscular volume (Fig. 7), mean corpuscular haemoglobin (Fig. 8) and mean corpuscular haemoglobin concentration (Fig. 9) were found to be low in some cases, suggesting iron-deficiency that may accompany PEM. Similar changes have also been reported in PEM by many workers from different parts of the world (Viteri, et al, 1968; Patwardhan et al, 1975; Verjee and Behal, 1976; Aggarwal et al, 1980).

Since protein is a major component of haemoglobin, it is plausible to expect that protein deficiency in PEM will produce a decrease in haemoglobin synthesis. The importance of dietary protein in the synthesis of haemoglobin is now well established. The
dietary deprivation of protein leads to a drop in haemoglobin concentration in a variety of experimental animals (Cartwright, 1948; Ghitis et al, 1963 a,b; Reissmann, 1964 a,b; Ito et al, 1964; Sood et al, 1965; Ito and Reissmann, 1966). The diminished formation of erythropoietin due to protein deficiency may also be a contributory factor in the anaemia of PEM since it leads to impaired erythropoiesis. The low level of erythropoietin leads to a drop in erythrocyte production, since reduced circulating reticulocytes, decreased iron utilization, and bone marrow hypoplasia have been reported in the anaemia of PEM.

It is known that the haemoglobin formation invariably takes precedence over the regeneration of plasma proteins, when proteins are realimented to anaemic and hypoproteinemic animals and human beings (Person et al, 1937; Hahn and Whipple, 1939; Ghitis et al, 1963 a,b; Reissmann, 1964 a,b; Sood et al, 1965). In the present study, however, the concentration of total plasma proteins returned to normal in both the groups following realimentation whereas the
haemoglobin levels were still lower as compared to controls in the first group and they were further reduced in the second group after dietary therapy. It seems that regeneration of plasma proteins is quite fast as compared to haemoglobin synthesis in PEM patients following high protein diet.

The other most important haemopoietic factor, apart from protein, is iron. The deficiency of iron is a common feature of PEM (Patel et al, 1965; Pereira and Baker, 1966; Manchanda et al, 1969; Bose, 1970; Lynch et al, 1970; Massa et al, 1978). The decreased availability of iron to the bone marrow in PEM may also lead to decrease in haemoglobin synthesis (Cronje et al, 1961). Moreover, it was found by Cronje et al (1961) that low levels of free erythrocytic protoporphyrin were associated with decreased $^{59}$Fe incorporation into the erythrocytes. It has been suggested that there may be an abnormality in protoporphyrin synthesis and/or a defect in iron transport in PEM.
There was a considerable increase in haemoglobin concentration in the first group after dietary rehabilitation with high protein diet along with minerals and vitamins. However, the haemoglobin levels were still significantly lower than those of controls (Fig. 4). This may partly be due to an increase in the plasma volume following rehabilitation. Insufficient rehabilitation period may be yet another factor responsible for persistent low levels of haemoglobin observed even after dietary therapy.

In the second group, the rehabilitation with the same diet but without iron, haemoglobin recovery was not as marked as in the first group (Fig. 4). Moreover, MCV and MCH values registered a decline (Fig. 7 and 8). The non-availability of iron for haemoglobin synthesis might be the reason for this observation. However, the observed small rise in haemoglobin concentration may be explained on the basis of the utilization of iron (whatever small amount is present in the body of these children as well as the iron present in the diet) during recovery. It may be said that the incomplete realimen-
tation might have led to the expression of otherwise latent deficiency of iron.

Viteri et al (1964) described partial improvement in the haematological parameters with milk alone in children suffering from severe PEM and this treatment induced the deficiencies of other haemopoietic factors, particularly of iron and folic acid (Kehta and Gopalan, 1956; Velez et al, 1963; Pereira and Baker, 1966). The majority of investigators conclude that in order to bring about a complete haematological recovery of PEM children protein, iron and folate must be given since most of the 'piggy back' deficiencies find less expression during this syndrome.

All the children in the control group had haemoglobin levels of 10 g/dl or more (Fig. 4). The peripheral blood picture showed normocytosis but mild hypochromia was also present in three children. The majority of the children in the control group showed normoblastic erythropoiesis. However, some degree of delay in haemoglobinization may account for mild
Fig. 10: Photomicrograph: Bone marrow smear showing megaloblastic erythropoiesis.

Fig. 11: Photomicrograph: Bone marrow smear showing iron-deficient erythropoiesis.
hypochromia observed in a few children. This may be due to low reticular iron present in the bone marrow of these children. This is in keeping with the consensus that sub-clinical iron-deficiency is quite common in apparently healthy subjects. In India, Patel et al. (1958) reported the incidence of iron deficiency to be 75 percent in apparently healthy infants and children. A large section of apparently healthy population in India is in the latent phase of iron deficiency, i.e., though the haemoglobin levels remain above the lower limits of the normal values, the bone marrow iron may be reduced (Cowan and Bharuch, 1973).

On the basis of peripheral blood smear morphology, the majority of children with PEM showed dimorphic anaemia with megaloblastic erythropoiesis and inadequate haemoglobinisation (Fig.10). However, microcytic hypochromic type of anaemia was also seen in a few children. The bone marrow morphology showed normoblastic erythropoiesis with inadequate haemoglobinisation in majority of the children and iron-deficient erythropoiesis (Fig.11) in some which...
Fig. 12: Photomicrograph: Peripheral blood smear showing microcytic hypochromic anaemia.
could account for microcytic hypochromic anaemia (Fig.12). It is apparent that the picture of anaemia in PEM is variable and this may be because of associated deficiencies of other haemopoietic factors and it has not been easy to define the effect of PEM per se on anaemia because of these deficiencies. These associated deficiencies may lead to varying degrees of nuclear and cytoplasmic abnormalities in the red cell during production and maturation (Viteri et al, 1968).

The bone marrow reticular iron grade was found to be reduced in majority of the children suffering from PEM. However, the marrow iron was absent in few cases. Majority of workers have observed that bone marrow iron is almost always present, though to a lesser extent, in uncomplicated cases of PEM (Kondi et al, 1963; Adams and Scrugg, 1965; Pereira and Baker, 1966).

There was a considerable improvement in peripheral blood and bone marrow morphology following
dietary rehabilitation in the first group. Nine children showed normoblastic erythropoiesis with adequate haemoglobinization and sufficient amount of iron in the bone marrow. The remaining six children presented with normoblastic erythropoiesis with inadequate haemoglobinization and decreased amounts of reticular iron stores. This may be because of the fact that these children had low bone marrow reticular iron on admission.

The rehabilitation regimen in the second group led to the development of microcytic hypochromic anaemia (Fig. 12). The bone marrow presented iron deficient erythropoiesis (Fig. 11) in majority of the children with iron grade either zero or traces. This may be due to the fact that the diet of these children was not supplemented with iron equivalent to total body deficit. Moreover, it has been reported that iron stores get depleted after dietary therapy (Kondi et al, 1965; Adams and Scragg, 1965; Adams et al, 1967). A few children also showed normoblastic
erythropoiesis with inadequate haemoglobinization and decreased amounts of iron present in the bone marrow.

The plasma iron concentration shows considerable circadian fluctuations, mainly due to varying amounts of iron release from reticuloendothelial (RE) cells (Fillet et al., 1974). Moreover, there are considerable day-to-day variations (± 30% or more) in plasma iron concentration (Bothwell et al., 1979). The plasma iron is mainly derived from RE cells and its levels vary according to marrow demand. It has been found that the plasma iron and iron present in the bone marrow may not run parallel since normal plasma iron concentration is associated with both reduced (Pirzio-Biroli and Finch, 1960) and greatly elevated (Rarker et al., 1968) amounts of iron in the marrow under different clinical conditions. The defective iron release from RE cells due to inflammation, liver disease, malignancy (Konijn and Mershko, 1977) or ascorbic acid deficiency (Lipschitz et al., 1971) will
depress plasma iron levels, as well as reduced transferrin concentration (Lahey et al., 1958). The low plasma iron level found in anaemia of chronic disorders may be due to defective mobilization whereas in iron-deficiency anaemia it may be because of exhaustion of mobilizable iron stores. It is invariably associated with increased amount of iron in the marrow in contrast to low marrow iron in the latter.

The fasting plasma iron levels were found to be significantly lower in PEM than those of controls of the same age groups (Fig.14). The majority of workers agree that PEM is commonly and invariably associated with a marked decrease in plasma iron concentration (Lahey et al., 1958; Edozien and Udzoeko, 1960; Adams and Scragg, 1965; Soothill, 1967; Antia et al., 1968; McFarlane et al., 1969; 1970,1972; El-Shobaki et al., 1972; Chattopadhyay and Banerjee, 1975; Reeds and Laditan, 1976; Brittenham et al., 1981). The decrease in plasma iron concentration observed in the present study may be explained on the basis of two most important factors operating simultaneously during
This syndrome. They are (a) diminished amount of iron present in the body resulting from insufficient intake and/or poor absorption and (b) decreased intake of protein resulting in reduced levels of iron carrier and storage proteins.

There is enough evidence that the iron intake is extremely low in these children as well as the absorption of iron is expected to be impaired since gastrointestinal disorders and protein malnutrition are two very common features of PEM. The low values of some of the gastric and pancreatic enzymes often observed in PEM (Thompson and Trowell, 1952; Schneider and Viteri, 1974; Mehta, 1979) may impair the iron absorption. The plasma iron levels have been found to be very low even in experimental protein deficiency in animals (Sood et al, 1965; Morgan and Peters, 1971). The decreased intake of protein in the diet and reduced levels of transferrin would also result in low absorption of iron (Klavins et al, 1959, 1963; Kroes et al, 1963; Fletcher and Muehns, 1968; Finch, 1975).

The acute protein deprivation in rats causes
erythroid hypoplasia in the bone marrow, associated with failure of iron utilization (Bethard et al., 1958 a,b; Reissmann, 1964 a,b). Many workers suggest that protein deficiency lowers the efficiency of iron utilization (Foy and Kondi, 1957; Klavins et al, 1963; Conard et al, 1967; Miski and Kratzer, 1977) and higher dietary protein is generally associated with enhanced iron uptake (Kuhn et al, 1968; Layrisse et al, 1974; Cook and Finch, 1975). In the present study the bone marrow iron was found to be reduced in majority of the children with anaemia of PEM. It is also believed that several free amino acids enhance iron absorption (Kroe et al, 1963; VanCampen and Gross, 1969; VanCampen, 1973) perhaps through chelation effect and also support the iron carrier protein synthesis hypothesis (VanCampen, 1972).

The primary function of transferrin is to transport iron from the cells involved in absorption and storage to other cells where iron is utilized for haemoglobin synthesis and incorporation into
cytochromes. A role for the transferrin molecule itself in regulating iron absorption has been proposed by Fletcher and Huehns (1968). Although iron absorption and transferrin saturation usually vary together, alterations in the latter do not seem to influence directly the changes in absorption. However, because of the extremely restricted iron losses in man, the absorptive mechanism becomes the regulator of iron balance. The man has been shown to have a system whereby a protein referred to as mucosal transferrin shuttles between lumen and mucosal cells to facilitate iron absorption (Huebers et al., 1975). The amount of this protein appears to be inversely related to individual's iron status (Savin and Cook, 1980).

The realimentation of the first group with complete diet showed marked increase in plasma iron concentration though the levels did not reach the control values (Fig. 14). The present duration of dietary therapy may be too short to expect full recovery. On the other hand, the rehabilitation of
second group led to a slight decrease in the plasma iron levels (Fig.14). This may be due to the increase in plasma volume following recovery. Moreover, the diet of these children was not supplemented with iron equivalent to child's body deficit as was done in the case of children in the first group. This may be yet another factor for observed fall in plasma iron levels after the stipulated dietary period of fifteen days. Moreover, the children of this group showed iron deficiency anaemia as indicated by low MCH and MCV, iron deficient erythropoiesis and microcytic hypochromic blood picture.

Normally there is an inverse relationship between plasma iron and total iron binding capacity (TIBC). Total iron binding capacity corresponds to plasma transferrin concentration, and unsaturated iron-binding capacity (UIBC) refers to that proportion of transferrin of which the binding sites are not already occupied by iron (Ramsay, 1973; Brittenham et al, 1981). In the present study both plasma iron and TIBC were found to be significantly low in subjects with PMA as compared to control (Fig.14).
and Fig. 15). According to Edozien (1960), Kwashiorkor is known to be associated with low plasma iron and low serum iron binding capacity. McFarlane and Soothill (1968) also observed similar findings with respect to TIBC and it was about 1/3rd of the normal level. Unpublished observations of Neale and Soothill (1958) confirmed that the iron binding capacity was down to about 1/3rd of the normal, but showed that transferrin (an iron binding protein) was much more profoundly depressed. Thus the low levels of TIBC could be due to decreased synthesis of iron binding protein (transferrin) due to less availability of protein. Unsaturated iron binding capacity was also found to be low in PEM group as compared to the control (Fig. 16) indicating the low level of transferrin in PEM.

The plasma transferrin levels have been found to be very low in children with severe PEM (Lahey et al., 1958; Edozien and Udoeze, 1960; Adams and Seragg, 1965; Soothill, 1967; Antia et al., 1968; Mc Farlane et al., 1969; 1070,1972; El-Sobaki et al.,1972; Reeds and Laditan, 1976; Brittenham et al.,1981) and
in experimental protein deficient animals (Sood et al., 1965; Morgan and Peters, 1971). Fasting and protein malnutrition also lead to markedly diminished rates of transferrin synthesis in rats (Morgan, 1969; Morgan and Peters, 1971) and albumin synthesis changes to a similar degree. Jeejeebhoy et al. (1973) have shown a 50 percent reduction in its rate of synthesis in protein deprived rats. It is probable that diminished transferrin levels found in PEM are due to decreased synthesis.

In man the major source of transferrin is the liver (Prunier et al., 1964; Gitlin and Biasucci, 1969). The decreased synthesis of transferrin could also result from impaired liver functions besides the non-availability of amino acids for its synthesis. Although the rate of synthesis of transferrin is inversely related to the hepatocyte ferritin (Mortan and Tavill, 1977, 1978) but is also altered by inflammation, hepatic dysfunction, malnutrition, pregnancy and oral contraceptives (Bothwell, et al., 1979). In PEM all the above mentioned factors might be playing a role in
reducing the transferrin levels and thereby leading to decrease in TIBC and UIBC.

There was a significant rise in TIBC and UIBC with dietary rehabilitation in both the groups (Fig.15,16), indirectly indicating that the transferrin levels might have improved with protein supplementation leading to increase in TIBC and UIBC. This fact has also been observed in children with Kwashiorkor in whom a rapid increase in transferrin concentration occurs after refeeding adequate diet (McFarlane et al, 1970). There was no significant difference in the levels of TIBC in the two groups following rehabilitation. The TIBC levels were still low as compared to the values reported in iron deficiency anemia. It may be because of the short duration of realimentation period.

The percent transferrin saturation, advocated by Bainton and Finch (1964), is a helpful parameter to differentiate iron-deficiency anemia from other conditions associated with iron deficiency. The percent transferrin saturation was found to be significantly
reduced in PEM as compared to the controls but it was not that low as reported in nutritional iron deficiency anaemia (Fig. 17). This could be due to low levels of plasma iron as well as transferrin. The dietary realimentation in the first group led to a significant increase in percent transferrin saturation since plasma iron levels and transferrin concentration had shown marked improvement (Fig. 17). However, percent transferrin saturation was significantly reduced in the second group following rehabilitation (Fig. 17). This may be because of low levels of plasma iron and elevated concentration of TIBC, a situation comparable to what is reported in pure iron deficiency anaemia.

However, percent transferrin saturation in the second group following rehabilitation was not that low as compared to what is generally observed in iron deficiency anaemia. This may be due to still lower TIBC in these children.

The total plasma protein as well as albumin levels are significantly reduced in established cases
of kwashiorkor but the alterations in marasmus have not been reported to be significant. This is because marasmus is an adapted condition to persistent calorie and protein deprivation. The serum protein levels are maintained at the expense of tissue proteins (Millward et al, 1975) because of adaptive hormonal changes (Gopalan, 1969). In chronic situation, there is a rise in free aminoacid pool in the liver; which results in increased protein synthesis. There is shift of albumin from extravascular compartment to the intravascular compartment for resynthesis (James and Hay, 1968). Moreover, the albumin catabolism has been found to be reduced (Cohen and Hanson, 1962).

The changes in serum protein in PEM pertain mainly to albumin. In normal children a serum albumin concentration of 3.5 g/dl or above is expected, values between 3.0 to 3.5 g/dl are considered subnormal and those below 3.0 g/dl are definitely low (Whitehead et al, 1971). In the present study, the total proteins in children suffering from PEM were observed to be
significantly below the control levels (Fig. 18). The dietary rehabilitation led to significant rise in their levels in both the groups and there was no significant different in the two groups (Fig. 18). Chatterjee and Mukherjee (1968), Dayal et al (1968) and Kalra et al (1980) have also reported subnormal serum protein levels in marasmus.

McFarlane et al (1969, 1970) and Gabr et al (1971) showed that low plasma transferrin concentrations were associated with poor prognosis in children with Kwashiorkor, and Reeds and Laditan (1976) extended this observation to marasmic children. Furthermore, the latter workers showed that serum transferrin concentrations were linearly related to deficit in weight and length for age in the under-nourished children although albumin concentrations were not. Others have also advocated the use of plasma transferrin concentration as a most sensitive index of PEM because of its rapid response to refeeding in malnourished children, and sensitivity to reduced protein intake.
in experimental animals (Oluci et al., 1975). However, the frequency with which iron deficiency occurs in malnourished children can complicate the situation to the extent that a decrease in transferrin concentration caused by malnutrition can be masked by an increase due to a lack of iron (Ismadi et al., 1971; Ingenbleek et al., 1975; Delpeuch et al., 1980).

**ERYTHROCYTE GLUTATHIONE METABOLISM IN ANAEMIA OF PEM**

Many biological oxidations proceed via formation of free radicals and/or peroxides and peroxidation by these is considered as a major contributor to the degenerative process that lead to ageing and cellular breakdown (Dormandy, 1969). This must be particularly true of the erythrocyte. The red cell, for several reasons, is extremely vulnerable to oxidative damage. First, it cannot replace inactivated proteins by new ones. Second, oxyhaemoglobin can participate in the generation of superoxide radicals
and hydrogen peroxide (Misra and Firdovich, 1972). Third, erythrocytes have no alternative means of generating NADPH other than the hexose monophosphate (HMP) shunt pathway. And finally, since red cells are under continuous control by the reticuloendothelial system, any deviation from the normal size or deformability will result in their removal from circulation.

The erythrocyte must have protective mechanisms against the oxidative stress (activated oxygen radicals and peroxides) which, if uncontrolled, could lead to oxidative damage of the cell (Carrell et al., 1975). It is for this reason that the erythrocyte shuttles much of its metabolic activity to reductive processes that maintain the adequacy of 'glutathione cascade' (Fig. 2). The glutathione cascade has been proposed to function as a metabolic unit in the reduction of peroxides (Reddy and Tapel, 1974). It consists of glucose-6-phosphate dehydrogenase (G-6-PD), reduced pyridine nucleotide coenzyme (NADPH), reduced glutathione (GSH), glutathione peroxidase (GSH-Px)
Reduced Glutathione (GSH)

The reduced glutathione (GSH) is a tripeptide of glutamine, cysteine, and glycine (L-$\gamma$-glutamyl-cysteinyl-glycine), and is a principal non-protein sulfhydryl compound present in many mammalian tissues (Jocelyn, 1972; Meister and Tate, 1976). The ubiquitous nature of GSH suggests that it plays important physiologic roles in virtually all cells. These roles include serving as a co-factor in certain enzymatic reactions (Raker, 1951; Strittmatter and Ball, 1955; Edwards and Knox, 1956), as well as participating in the $\gamma$-glutamyl cycle (Meister, 1973).

The glutathione accounts for over 95 percent of the reduced non-protein sulfhydryl compounds present in the erythrocytes (Woodward, 1955; Michel, 1944; Beutler et al, 1963). It is known that neither
oxidized glutathione (GSSG) nor GSH can enter the red
cell (Eldjarn et al, 1962; Horejsi and Mircevova, 1964)
although GSSG has been shown to leave the cell (Srivastava
and Beutler, 1967, 1969). Therefore, the glutathione with­
in the human red cell must be replaced through de novo
synthesis and the synthesis has been demonstrated since
the enzymic machinery necessary to synthesize GSH from
the three constituent amino acids is present in the
mature erythrocytes (Jackson, 1969; Majerus et al, 1970).
Moreover, glutathione is also maintained in its func­
tional reduced form (GSH) by a specific enzyme, gluta­
thione reductase whose preferred cofactor is NADPH
(Conn and Vennesland, 1951; Mapson and Goddard, 1951;

The GSH molecule has two interesting chara­
ccteristics: (a) a \( \gamma \)-glutamyl linake, and (b) a
highly reactive reduced sulfhydryl (\(-SH\)) group. The
\(-SH\) group of GSH provides reducing power for the cell.
The red cell, for several reasons as mentioned
previously, is extremely vulnerable to oxidative damage
and GSH plays a very important role in maintaining the integrity of this rather 'disadvantaged cell' by keeping the sulfhydryl groups in the reduced form within the red cell and perhaps on the red cell surface (Allen and Jandl, 1961; Jacob and Jandl, 1962 a,b,c). GSH has also been found to be necessary for the stability of many red cell proteins including several enzymes (Woodward, 1935; Barron and Singer, 1943; Rapport and Schench, 1960), coenzymes (Schench et al, 1961) and haemoglobin (Jandl and Allen, 1960; Schench et al, 1961; Allen and Jandl, 1961; MacDonald and Huisman, 1962). Thus GSH is involved in shielding essential sulfhydryl enzymes against inactivation, guarding membrane lipids against peroxidation and protecting haemoglobin against irreversible oxidative denaturation. The GSH very well fulfils this protective function by being more readily oxidized than the vital cellular constituents (Crock, 1959; Rapport and Schench, 1960) or at least by promptly reversing the earlier oxidative changes in them (Beutler, et al, 1957).

The concentrations of GSH have been expressed
as mg/dl of whole blood, mg/g Hb and $1 \times 10^{-8}$ μg/RBC in the present study. It is generally believed that main function of GSH is to maintain stability and function of haemoglobin by keeping its -SH groups in the reduced form (Jacob and Jandl, 1962; Jacob et al., 1968). This indicates the advantage of expressing GSH level in relation to haemoglobin concentration since any change in GSH concentration in relation to haemoglobin will be significant for the above mentioned function of GSH. Although GSH has very important role to play in relation to haemoglobin as stated above, it is also required for the stability and optimal function of the sulfhydryl dependent enzymes present in RBC as well as for maintaining the integrity of red cell membrane. Thus it may be of advantage to express GSH concentration as $1 \times 10^{-8}$ μg/RBC and the author of the present study is strongly in favour of this method of expression.

The levels of GSH in anaemia of PEM were found to be significantly low when compared with the controls. The percent decrease (50.2%) was maximum when GSH concentration was expressed as mg/dl of
whole blood (Fig.19). But this expression may simply mean a reduction in the number of RBC and may not have any relevance in relation to the reasons for anaemia. However, when levels were expressed as $1 \times 10^{-8} \mu g/RBC$ (Fig.20), the reduction in the PEM group was about 20.3 per cent and the reduction is least (9.4%) when expressed as mg/g Hb(Fig.21).

Thus in the present study the erythrocytes from patients of PEM have subnormal levels of GSH. There are several factors which have been shown to influence the concentration of GSH in tissues and in blood (Theil et al, 1961; Goswitz et al, 1966; Hsu et al, 1968; MacDougall, 1968; Lambert and Thorgeirsson, 1976; Abraham et al, 1978; Tateishi and Higashi, 1978). Of all, the diet seems to play an important role in maintaining the concentration of GSH since in nutritional deficiency syndromes the GSH concentration is reported to be reduced (Verjee and Behal, 1976; Fondu et al, 1978 d).

The decrease in GSH contents of the red cell in PEM could be related to the non-availability
of the constituent amino acids and the deficiency of which have been reported in this syndrome (Ghisolfi et al, 1978; Worthington et al, 1979). It has also been observed in rats that protein deprivation reduced the levels of GSH to 20 per cent in the liver (Lindan and Work, 1953). It is, therefore, reasonable to suggest that protein deficiency or even starvation will result in the decreased endogenous synthesis of this tripeptide. This fact has been substantiated by observing a rapid decrease in rat liver GSH concentration during starvation and its quick reappearance upon refeeding (Binet and Weller, 1935; Leaf and Neuberger, 1947; Edwards and Westerfeld, 1952; Register et al, 1959; Maruyama et al, 1968; Tateishi et al, 1974; 1977; Higashi et al, 1977; Cho et al, 1981).

Often the GSH levels have been shown to be increased in other nutritional anaemias particularly due to iron or vitamin B₁₂ deficiency (Varela et al, 1931; Bach and Bach, 1931; Michel, 1944; Viviani et al, 1956; Lawrence and Grossman, 1965; Hopkins and Tudhope, 1973). Although some contradictory reports
are also available (Ling and Chow, 1953; Register, 1954; Hsu et al, 1959; Jocelyn, 1960; O'Dell et al, 1961). These raised levels are said to be required to protect the erythrocytes from further oxidative damage. Because of the low levels of GSH, the erythrocytes of PEM patients are likely to be exposed to greater risk of oxidative damage compared to the erythrocytes of pure iron or pure vitamin B₁₂ deficiency anaemia.

Verjee and Behal (1976) have observed dramatic increase in GSH concentration on rehabilitation in patients with anaemia of PEM and at times the levels may overshoot the control values (Lindan and Work, 1953). In the present study GSH levels improved significantly following dietary rehabilitation of the first group in which the diet was complete in all respects and the iron deficit was completely replenished (Fig.19,20,21). The levels, however, did not reach the control values. This may be due to the duration of rehabilitation period which was comparatively less than what was used by Verjee and Behal (1976).
In the first rehabilitation group, haemoglobin and GSH increased almost in a parallel fashion. We observed a significant positive linear correlation of GSH concentration (mg/g Hb) with haemoglobin levels as is obvious from the regression lines in the control group (Fig. 22) and in the first group following realimentation (Fig. 23). The parallel increase of GSH and haemoglobin during rehabilitation of the first group may be because of the fact that the requirements for haemoglobin regeneration (amino acids, iron and other haemopoietic factors) as well as for GSH synthesis (amino acids) are properly met with. The regression line, in first group after dietary treatment, indicate that for a slight rise in the Hb., there is a considerable increase in GSH level.

Although we have found an almost parallel increase in GSH and haemoglobin in the first rehabilitation group, no perfect parallelism is seen when we compare haemoglobin and GSH levels in patients of PEM. This may be explained on the basis that the anaemia of PEM is multifactorial and haemoglobin
regeneration depends upon a number of dietary haemopoietic substances, the deficiency of which have been shown to accompany this syndrome (Verjee et al, 1975) while GSH synthesis mainly requires a proper amino acid pool. Thus the failure of parallelism in patients of PBIL may be due to variable deficiency of haemopoietic factor and protein.

The rise of GSH (mg/g RBC) after dietary therapy (Fig. 21) is much more in the second group (56.1 per cent) as compared to the first group (13.3%). The percent increase of GSH as 1x10^-8 μg/RBC is also more (Fig. 20) in this group (20.1%) in relation to the first group (15.2%). The reduced glutathione levels were also found to be higher significantly (when expressed as mg/g Hb) in this group following rehabilitation as compared to the controls (Fig. 21) although haemoglobin concentration did not improve much and they were still significantly below the control values. There may be different reasons for more rapid synthesis of GSH than haemoglobin during reha-
bilitation of the second group. As already mentioned, the GSH regeneration needs only a proper amino acid pool. The amino acid pool could be expected to be optimal with adequate protein intake in this group during dietary therapy. The haemoglobin regeneration on the other hand requires, besides a proper amino acid pool, other haemopoietic factors including iron. Since iron deficit was not compensated in this group, regeneration of haemoglobin cannot be expected to be optimal.

As mentioned above, the children in second group did not much improve their haemoglobin levels and many of them actually developed frank iron deficiency anaemia (page 164). The relative rise of GSH in nutritional iron deficiency anaemia and other types of anaemia has been reported by many workers (Lawrence and Grossman, 1965; MacDougall, 1968; MacDougall et al, 1970; Hopkins and Audhope, 1973 a). It could thus be argued that in the second rehabilitation group, as the protein and caloric intake was normalized, the usual tendency on the part of RBC to increase their status of GSH in order to
pooteot the low levels of haemoglobin in the anaemic cells became manifested. This also brings to light the probable reason for low GSH levels in anaemic erythrocytes of P3M.

It is well known that the sulfhydryl groups of haemoglobin need protection from oxidative damage and this protection is provided by GSH. It has also been reported, however, that the sulfhydryl groups of haemoglobin have a protective function in relation to other-SH-containing components of erythrocytes. The reduction in the haemoglobin levels would result in a decrease in the reactive sulfhydryl groups. With the finding of increased GSH concentration, it might be tempting to speculate that this was designated to compensate for lack of the haemoglobin sulfhydryl groups (MacDougall, 1968). The increase in GSH concentration, however, has been reported in a number of clinical conditions associated with normal haemoglobin levels.

Apart from the role of protecting haemoglobin from oxidative denaturation (Allen and Jandl,
1961), GSII is known to play an important role in maintaining the integrity of red cell membrane (Benesch and Benesch, 1954; Jacob and Jandl, 1962 a,b,c) and the stability of the sulfhydryl dependent enzymes (Schench et al, 1961; Eldjarn et al, 1962). The sulfhydryl dependent enzymes have been shown to be increased in nutritional iron deficiency anaemia (MacDougall, 1968). Therefore, an associated increase in GSII concentration may be required to ensure optimal enzyme functions.

The levels of GSII in the red cells are also maintained by de novo synthesis from the amino acid pool and this aspect has been discussed in the preceding pages. The GSII levels would, however, also depend upon its regeneration from oxidized glutathione (GSSG) by the activity of NADPH dependent GSSG-R. The NADPH level in turn is maintained by the reduction of NADP+ by HMP shunt enzymes, viz., glucose-6-phosphate dehydrogenase (G-6-PH) and 6-phosphogluconate dehydrogenase (6-PG-d). The deficiencies of GSII may result from either genetic or secondary deficiencies of any
of these enzymes of glutathione metabolism. The deficiencies of these enzymes can also be expected in PEM. In fact, decreased activities of GSSG-R and G-6-PD have been reported in PEM (Verjee and Behal, 1976; Fondu et al, 1978) which have been discussed along with our results of the present study (page 207).

The reduced glutathione exerts its protective action in various ways; either directly as a free radical scavenger by competing with other SH-carriers or by means of repair reactions via mixed disulfide formation or acting as a substrate for GSH-Px, i.e., by removing peroxide enzymatically. Thus GSH offers protection to erythrocytes against oxidative stress by maintaining the stability of sulfhydryl containing enzymes(s) and haemoglobin besides mediating the detoxification of peroxides and hydroperoxides. It clearly indicates that in the red cell GSH is vital in the maintenance of its normal infrastructure under physiological conditions by maintaining the reduced environment within the cell. It can, therefore, be
Reaction-1
Lipid peroxides and hydroperoxides.
safely said that the erythrocytes, already deficient in GSH during PEM syndrome, may be in a precarious situation since in PEM there is likelihood of deficiencies of other cellular antioxidants such as ascorbic acid and vitamin E. Thus decreased levels of GSH in PEM may contribute to the shortened survival of RBC in this syndrome.

**Glutathione Peroxidase (GSH-Px)**

The red cell glutathione peroxidase (glutathione: \( \text{H}_2\text{O}_2 \) oxidoreductase; E.C.1.11.1.9; GSH-Px) is an enzyme functionally connected with the hexose monophosphate (HMP) shunt pathway. Mills (1957, 1959) first demonstrated the presence of this enzyme in the mammalian red cells which plays a strategic role in the protection of the erythrocytes from oxidant stress. The presence and importance of GSH-Px in the human red cells has subsequently been clearly outlined by Cohen and Hochstein (1963) and Hill et al (1964). It was demonstrated that this enzyme represents, under physiologic conditions, the major pathway in catabolizing hydrogen peroxide and lipid peroxides/hydroperoxidase (reaction 1).
and thereby preventing these agents from initiating cellular damage (Little and O'Brien, 1968; Chow et al, 1973; Rotruck et al, 1973; Hoekstra, 1975). It thus helps in maintaining the integrity of the erythrocytes by preventing the oxidation of the sulfhydryl (-SH) groups of haemoglobin, membrane proteins and cellular enzymes. It is, consequently, obvious that GSH-Px deficiency, if sufficiently severe, may cause premature erythrocyte loss.

Moderate or severe deficiency of erythrocytic GSH-Px is now well recognized as a genetically determined enzymopathy. The clinical expression of this enzyme deficiency is often accompanied by a variable degree of haemolysis (Necheles et al, 1968, 1969, 1970; Boivin, 1971; Steinberg and Necheles, 1971; Necheles, 1973; Tursz et al, 1974). In addition to genetic polymorphism, a number of environmental variables including nutritional factors have been shown to influence the GSH-Px activity and/or synthesis. It, therefore, seems probable that multiple factors may be involved in modulating the activity of GSH-Px and/or synthesis of active enzyme.
molecules during the development of an erythrocyte. The nutritional factors such as selenium (Noguchi et al., 1973; Chow and Tappel, 1974; Thomson et al., 1977; Perona et al., 1977 b), iron (Rodvien et al., 1974; Cellerino et al., 1976), vitamin E (Yang and Dessai, 1978) and feeding of lipid peroxides (Chow et al., 1973) have been found to influence the activity of GSH-Px.

A decrease in red cell GSH-Px activity, even in the absence of any overt sign of haemolysis, has been observed in clinical cases of iron-deficiency in man (MacLoughlin, 1972; Hopkins and Tudhope, 1973 b; Cellerino et al., 1976; Perona et al., 1977 b) and in iron-deficient experimental animals (Rodvien et al., 1972, 1974). On the other hand, erythrocytes from individuals with megaloblastic anaemia due to vitamin B\textsubscript{12} deficiency have higher GSH-Px activity than normal and there is a fall in the enzyme activity following vitamin B\textsubscript{12} therapy (Hopkins and Tudhope, 1973 b). The patients with heterozygous β-thalassaemia exhibit increased erythrocyte GSH-Px activity even though
Haemoglobin and MCH values are similar to those observed in iron-deficiency anaemia (Cellerino et al, 1976). Thus the changes in erythrocyte GSH-Px activity do not necessarily relate to either red cell number or haemoglobin concentration. Moreover, it does not appear to relate to the differences in the mean age of red blood cells because the enzymatic levels do not differ significantly between reticulocyte-rich and poor fractions (Cellerino et al, 1976).

The compensatory increase in GSH-Px activity has also been observed in response to disease or to the deficiency of nutritional factor such as vitamin E. This type of compensatory increase has been found to occur in G-6-PD deficiency (Beutler, 1977a), α-thalassemia syndrome (Beutler, 1977b; Zimmerman and Natta, 1981), sickle cell anaemia (Zimmerman and Natta, 1981), vitamin E deficient experimental animals (Chow et al, 1973; Vilas et al, 1976) and even during direct exposure to peroxides (Vilas et al, 1976). All the above mentioned conditions are believed to lead ultimately to increased
cellular oxidative process (Beutler, 1977 b) and thus a compensatory increase of GSH-Px is helpful for the erythrocyte against the oxidative damage.

The low levels of vitamin E have been shown to occur in some types of haemolytic anaemias in association with increased sensitivity to hydrogen peroxide-induced haemolysis in vitro (Rachmilewitz et al, 1976). Recently Zimmerman and Natta (1981) reported a significant increase in GSH-Px activity compensatory to the decrease in plasma tocopherol levels in sickle cell anaemia. Natta and Machlin (1979) and Chiu and Lubin (1978) also reported elevated levels of GSH-Px in sickle cell anaemia along with low levels of vitamin E. On the other hand, normal GSH-Px levels have also been reported in vitamin E deficiency (Scott et al, 1976; Yang et al, 1976). Yang and Desai (1976) have demonstrated the inter-relationship between erythrocytic GSH-Px and vitamin E status. Thus, it is reasonable to conclude that the response of GSH-Px to vitamin E status may be variable.
It was observed in the present series of patients that the anaemia of PEM is accompanied with a significant decrease in red cell GSH-Px activity whether expressed as IU/gHb or IU/10^10 RBC as compared to the controls (Fig.24 and 25). Verjee and Behal (1976) and Fondu et al (1978) also reported significant reduced activity of erythrocyte GSH-Px in patients with PEM. The decreased activity of this enzyme in PEM could be explained on the basis of a number of nutritional factors already mentioned.

The decreased GSH-Px activity in PEM may be because of less synthesis of apo-protein enzyme molecules, during the development of an erythrocyte, due to poor amino acid pool. In addition to protein, iron deficiency, as observed in these children, may also be held responsible for the decreased activity of GSH-Px observed in the anaemia of PEM. The erythrocytic GSH-Px showed a positive linear correlation with plasma iron concentration and haemoglobin level both in the control group (Fig.26,27) as well as in the children of first group on admission and after two
weeks of treatment (Fig.28,29). Cellerino et al (1976) also observed a linear positive correlation of red cell GSH-Px activity with plasma iron concentration. However, it is not yet clear, what role iron is playing in modulating the activity and/or synthesis of this enzyme since heme is not a structural component of the enzyme. Rodvien et al (1974) suggested that iron may be necessary for the enzyme to be synthesized during the development of red cells within the marrow or a non-heme iron containing protein serving as an electron carrier or coenzyme may be required for the GSH-Px to function.

The deficiency of selenium which is likely to be present in these children, may also be responsible for the decreased GSH-Px activity since it is a selenoenzyme. The selenium has been shown to be an integral part of erythrocytic GSH-Px (Rotruck et al, 1973; Hoekstra et al, 1973; Flohe et al, 1973) and recent observations suggest that it has 4 selenium atoms in each molecule (Flohe, 1971; Flohe et al, 1973; Oh et al, 1974; Awasthi et al, 1975). It has been reported that the availability of selenium in the bone marrow also affects
the levels of GSH-Px (Fondu et al, 1978 a; Perona et al, 1978). Moreover, the activity of GSH-Px has been shown to be directly related to the availability of dietary selenium (Chow and Tappel, 1974; Hafeman et al, 1974; Smith et al, 1974) and a close positive correlation has been found between selenium levels and GSH-Px activity both in plasma and in red cells (Thomson et al, 1977; Rudolph and Wong, 1978).

The animal feeding experiments have also demonstrated a good correlation between dietary selenium intake and activity of GSH-Px in various tissues (Chow and Tappel, 1974; Hafeman et al, 1974; Smith et al, 1974; Tappel, 1974). The decreased synthesis of the enzyme in erythroid precursors, because of less availability of protein and selenium, may be responsible for the decreased activity of GSH-Px in PEM.

It has been postulated by Perona et al (1978) that besides helping synthesis of GSH-Px in the bone marrow erythroblasts selenium activates this enzyme allosterically. This is good indication that
the selenium stores may be exhausted in PEM (Burk et al, 1967). The decreased plasma and whole blood selenium contents have been reported in PEM (Schwartz, 1965; Majaj and Hopkins, 1966; Burk et al, 1967; Levine and Olson, 1970). Furthermore, it has also been observed that the children with this syndrome benefit from selenium administration (Schwartz, 1961; Hopkins and Majaj, 1967).

The rehabilitation of the first group with complete diet led to a significant increase in GSH-Px but the activity did not reach the control value (Fig. 24,25). The observed increase in GSH-Px may be because of the fact that all those factors which are known to affect this enzyme have been compensated following dietary therapy. The erythrocytic GSH-Px showed a close correlation with haemoglobin level and plasma iron concentration in the control group (Fig.26,27), an observation analogous to what is observed in patients with PEM on admission (Fig.28,29) and even after two weeks dietary therapy (Fig.28,29). This also supports the importance of iron for the optimal synthesis and/or activity of GSH-Px.
It may, however, be noted that GSH-Px activity has shown an increase even when expressed as IU/gHb. This means, although, haemoglobin and GSH-Px both must have increased with increase in plasma iron concentration during rehabilitation of the first group, increase in GSH-Px activity must have been much more so that its level expressed as IU/gHb has also increased. It thus appears that relation between plasma iron and red cell GSH-Px is more sensitive than between plasma iron and haemoglobin. It is interesting to note that in iron-deficiency, the levels of both haemoglobin and GSH-Px are decreased but the reduction of GSH-Px is more significant as compared to decrease in haemoglobin level. Similarly when iron is provided in the diet along with other food nutrients, the levels of both the molecules increased, but the improvement is much more in GSH-Px activity than in haemoglobin level.

This sensitive relationship between iron and GSH-Px is illustrated by the results of second group following realimentation (Fig.24,25). We know that in this group the regeneration of haemoglobin
was much less as compared to the first group because of critical iron-deficiency. But the decrease in GSH-Px (IU/gHb) compared to the control value explains the more sensitive relationship between iron and this enzyme. The results are diametrically opposite to those of GSSG-R, the levels of which increased to a greater extent and those of haemoglobin to a lesser extent in the second group as compared to the first one. This led to much more increase in the levels of GSSG-R in the second group compared to the first when the results are expressed as IU/gHb.

The reduction in the activity of GSH-Px (IU/gHb) during dietary treatment in the second group may also mean that critical role of iron in the activity and/or synthesis of this enzyme becomes more exaggerated when other deficiencies are compensated. This is a situation similar to the one noted earlier that element of iron-deficiency becomes more clearly visible when other deficiencies are replenished during realimentation in the second group.
We have referred above to the rapid regeneration of GSSG—H when deficiency of all the nutrients but iron was compensated. This increase has been explained as a compensatory mechanism on the part of erythrocyte so as to protect the haemoglobin against oxidative damage and that this compensatory mechanism comes into play only when all other nutrient deficiencies are replenished. It is, however, surprising that this type of compensation is not available in relation to GSH-Px which in no way is less important an enzyme involved in oxidant neutralization.

It has been demonstrated that severe iron-deficiency anaemia both in man and in experimental animals is associated with reduced GSH-Px activity (MacDougall, 1972; Hopkins and Tudhope, 1973b; Rodvien et al, 1972, 1974; Cellerino et al, 1976; Perona et al, 1977 a) and supplementation of iron caused a rapid increase in GSH-Px (MacDougall, 1972; Hopkins and Tudhope, 1975 b; Rodvien et al, 1974). The diminished GSH-Px activity has been implicated as a possible mechanism of shortened RBC survival in iron-deficiency anaemia.
The studies in iron-deficient human subjects suggested that decrease in GSH-Px parallels the decrease in haemoglobin level (MacDougall, 1972; Hopkins and Tudhope, 1973b), while a study with iron-deficient rabbits demonstrated that the decrease in red cell GSH-Px activity was manifested even when expressed per unit of haemoglobin (Rodvien et al, 1974). The results of the present study that erythrocytic GSH-Px has a good correlation with haemoglobin support the above mentioned observation.

Anaemia or hypochromia per se in iron-deficiency anaemia does not appear to be responsible for diminished activity of GSH-Px in iron-deficiency anaemia since the anaemia induced by bleeding or phenylhydrazine does not lead to a decrease in GSH-Px activity (Rodvien et al, 1974). Moreover, pernicious anaemia (a conditioned vitamin B₁₂ deficiency) (Hopkins and Tudhope, 1973b) and heterozygous ψ-thalassaemia (Cellerine et al, 1976) in man was associated not with a decrease but by an increased levels of erythrocytic GSH-Px even though
haemoglobin levels and MCH values were similar to those observed in patients with severe iron-deficiency anaemia. Thus the changes in erythrocytic GSH-Px activity do not necessarily relate to either red blood cell number or haemoglobin concentration. Although our findings in PEM are similar to those of iron-deficiency anaemia, the situation is quite different with respect to the aetiology of anaemia of PEM and protein deficiency is only one component of a complex haematological picture in which vitamins (particularly vitamin B₁₂ and tocopherol) and minerals (especially iron and selenium) deficiencies also play prominent roles.

It has been observed that there is an increased vulnerability of haemoglobin to irreversible oxidative denaturation by peroxides whenever the capability of GSH-Px was impaired due to either a genetic deficiency of the enzyme itself (Necheles et al, 1965; Necheles, 1974) or due to the decreased activity because of the non-availability of environmental factors such as
iron, selenium, vitamin E (Dipllock, 1978; Perona et al., 1978; Yang and Desai, 1978) and an inadequate supply of its cofactor, i.e., GSH (Carson et al, 1956; Oort et al., 1961; Brewer and Dern, 1964; Lohr, 1964; Hochstein, 1971; Boivin et al, 1974; Lohr et al, 1974). However, to date there is no convincing evidence for a cause-and-effect relationship between enzyme activity and haemolytic anaemia as such.

In the anaemia of PEM, there are many limiting factors, two of them seem to be most important in relation to the protection of erythrocytes against oxidative damage. The first, a reduced GSH-Px activity with a consequently decreased ability to detoxify peroxides, and the second a markedly low levels of haemoglobin available for buffer action to protect membrane lipids. Since the two factors co-exist to a sufficient degree besides the likely deficiency of antioxidants particularly of tocopherol (Majaj et al, 1963; Majaj, 1966; Whitaker et al, 1967; Halstead et al, 1969), it is obvious that the red cell during PEM syndrome may
be more vulnerable to haemolytic agents and oxidative drugs or even under basal conditions. In this way we could explain the shortened erythrocyte survival and decreased red cell fragility observed in anaemia of PEM (Lanzkowsky et al, 1967; Brown et al, 1978).

**GLUTATHIONE REDUCTASE (GSSG-R)**

Red blood cell glutathione reductase (GSSG-R; NADPH: GSSG oxidoreductase; E.C.1.6.4.2) catalyses the reduction of 1 mol of GSSG to 2 mol of GSH with the simultaneous oxidation of NADPH to NADP⁺ (Reaction I).

\[
\text{GSSG + NADPH + H}^+ \xrightarrow{\text{FAD}} \frac{\text{GSSG-R}}{\text{GSH + NADP}^+ (1)}
\]

This enzyme has been the subject of extensive study and it is generally accepted that GSSG-R is largely responsible for the maintenance of intracellular glutathione in the reduced form (GSH). This enzyme also functions intracellularly in the reduction of mixed disulphides.
of glutathione and proteins, e.g. GS-Hb in erythrocytes and GS-crystallin in the lens (Srivastava and Beutler, 1970, 1973; Beutler, 1974; Beutler and Srivastava, 1974).

GSSG-R has been reported to be a flavoprotein with FAD as the prosthetic group and requires this riboflavin containing cofactor for optimum enzymic activity (Buzard and Kopko, 1963; Scott et al, 1963; Colman and Black, 1965; Icen, 1967; Staal et al, 1969a; Srivastava and Beutler, 1970). Beutler (1969a) has shown that the NADPH-methemoglobin reductase and the NADPH-glutathione reductase are the only enzymes in the erythrocytes requiring FAD. The enzyme GSSG-R is present in at least two forms, an active form associated with FAD and an active form unsaturated with respect to FAD (Icen, 1967; Beutler, 1969b; Staal et al, 1969a,b; Yawata and Tanaka, 1974). It has been found that the activity of red cell GSSG-R from apparently normal subjects is strongly stimulated in vitro by minute quantities of FAD and in vivo by administration of physiological amounts of riboflavin (Beutler, 1969c). During GSSG-R assay, addition of
FAD to the assay medium activates the inactive form and the degree of activation has been used as an index of riboflavin status in man (Glatzle et al, 1968; Bamji, 1969; Beutler, 1969a; Glatzle et al, 1970; Tillotson and Sauberlich, 1971; Bamji and Sharada, 1972).

The literature contains numerous reports of wide variety of syndromes associated with diminished GSSG-R in the red cells. Beutler (1969b,c) observed that in great majority of the cases and even in some normal subjects, apparent GSSG-R deficiency may not be related to the apoenzyme itself but to the sub-optimal amounts of its cofactor, flavin adenine dinucleotide (FAD). The latter is synthesized from dietary riboflavin and therefore depends upon riboflavin nutrition and metabolism (Bamji, 1969; Beutler, 1969b,c; Flatz, 1970, 1971a,b,c). It has also been demonstrated that the activity of GSSG-R could be restored by the addition of FAD in vitro or by administration of riboflavin in vivo (Beutler, 1969b; Staal et al, 1969a,b; Benohr et al, 1973a,b). However, the absolute genetic deficiency of GSSG-R did not show any restoration of activity when
FAD was added to the red cell GSSG-R assay system or when the patient’s erythrocytes were re-examined after riboflavin rich diet (Loos et al, 1976). It seems therefore reasonable to say that the enzyme defect was different from the nutritionally induced GSSG-R deficiencies described in literature.

The activity of GSSG-R in the mature erythrocytes depends upon the rate of synthesis of the apoprotein enzyme molecules in the reticulocytes which in turn depends upon the proper amino acid pool. Moreover, red cell GSSG-R activity is also influenced by the plasma levels of riboflavin and the activities of the enzymes responsible for the conversion of riboflavin into FAD. The plasma flavin levels have been shown to determine the red cell contents of this vitamin (Burch et al, 1948). and it has also been reported that extracellular riboflavin can be incorporated into erythrocytes and converted to FAD enzymatically (Mandula and Beutler, 1970).

The erythrocytic GSSG-R activity, IU/g Hb or IU/1x10^{10} RBC) was found to be significantly low in children
with PEM as compared to the controls (Fig.30,31). The results of the present study are comparable with those reported by Verjee and Behal (1976) and Fondu et al (1978 c). The reduced activity of GSSG-R in PEM could be explained on the basis of decreased synthesis of apoprotein enzyme molecules for GSSG-R and for FAD forming enzymes because of poor amino acid pool or because of less availability of riboflavin, as patients with PEM are likely to be associated with the deficiency of riboflavin (Poy et al, 1961; Kondi et al, 1963). The deficiency of the flavin metabolizing enzymes in PEM (flavokinase and FAD pyrophosphorylase) could result in the diminished synthesis of FAD from riboflavin thereby regulating the degree of association of GSSG-R with FAD in the red cells. However, Verjee and Behal (1976) observed that the addition of FAD to the assay system caused a slight inhibition of GSSG-R activity in PEM. It is, therefore, possible that the behaviour of this enzyme to normal FAD stimulation is different in PEM from that of sub-optimal riboflavin nutrition alone. Thus, the activity of GSSG-R in the red cells of PEM patients might be low because of poor
amino acid pool and also might be controlled by some additional unknown mechanism other than flavin metabolism regulating the enzyme synthesis or activity.

Children in both the groups showed a significant increase in GSSG-R activity following dietary therapy (Fig. 30, 31). The increase in the activity of GSSG-R in the second group was much more than in the first group if it was expressed as IU/g Hb (Fig. 30). In the second group, the final values exceeded even those of the control group. This apparently means that compared to the increase in haemoglobin concentration, there is a greater increase in the activity of GSSG-R in the second group. In fact, the rehabilitation of the second group led to a decrease in MCH values meaning thereby that the patients have developed iron-deficiency type of anaemia. As already mentioned whatever improvement is observed in haemoglobin concentration (g/dl) in the second group can be explained on the basis of increase in RBC count.

The above referred much greater improvement
in the cellular levels of GSSG-R compared to hemoglobin regeneration may be explained on the basis that the requirements for synthesis and activity of this enzyme, i.e. a proper amino acid pool and riboflavin have been provided in the regimen of the second group. On the other hand, the requirement of iron which is specific and absolute for the regeneration of hemoglobin was not replenished in this group. Iron-deficiency appears to have become a more limiting factor for the formation of hemoglobin when the deficiencies of other nutritional food factors have been compensated with the diet becoming normal.

As pointed out above after rehabilitation in the second group the anemia became pure iron deficiency type and with the compensation of other deficiencies, it became possible for the body to bring up the protective mechanism against oxidative denaturation of hemoglobin in the form of increase erythrocytic glutathione reductase levels. The present observation of increased GSSG-R activity (IU/gHb) in the second group following dietary realimentation is analogous
to that reported by other workers in iron and/or vitamin B$_{12}$ deficiency anaemia (Hopkins and Tudhope, 1973 b; Ramachandran and Iyer, 1974). This is true since increased GSH concentration has to be maintained in these conditions to protect the haemoglobin molecule from oxidative denaturation. This point has been discussed in detail on page 187.

The activity of GSSG-R may vary within wide limits without any increased susceptibility to oxidant challenge occurring. Beutler and Srivastava (1970) have reported that large (non-hereditary) reductions of GSSG-R activity in rats and in humans are neither associated with decreased red cell survival, nor with abnormal sensitivity to oxidant drugs. Furthermore, relative (primarily non-hereditary) GSSG-R deficiency does not seem to exacerbate the haemolytic response to various oxidants (Carson et al, 1981; Beutler and Srivastava, 1970). These last authors as well as Paniker et al (1970) have concluded that a reduction in GSSG-R activity upto 50 per cent of normal is not rate limiting in protection against oxidative damage to red cell.
Beutler and Srivastava (1970) were unable to find any enhanced susceptibility to haemolytic anaemia in GSSG-R deficient rats or in human subjects. Recently, Beutler (1979) concluded that partial deficiency of GSSG-R, whether nutritional or hereditary in origin, has no known haematologic effect. However, in GSSG-R deficiency of genetic origin, there are several reports of increased haemolytic response to oxidant agents (Carson et al., 1961; 1963; Waller et al., 1964; Carson and Frischer, 1966; Waller, 1967). Waller (1968) has observed that GSSG-R deficient red cells consistently show increased Heinz body formation upon incubation with acetylphenylhydrazine. This probably can be traced to a diminished rate of GSSG-R dependent reduction of mixed disulfides which are formed during oxidant stress. This diminished reduction would result in the accumulation of haemoglobin molecules with extensive sulfhydryl oxidation. A GSSG-R deficiency, which apparently caused non-spectrocytic haemolytic anaemia, was first reported by Lohr and Waller (1962).
Gluco-6-phosphate dehydrogenase (G-6-PD)

Gluco-6-phosphate dehydrogenase (glucose-6-phosphate: NADP oxidoreductase; E.C.1.1.1.49; G-6-PD) is the initiating and primary enzyme of the alternate oxidative pathway for glucose (hexose mono-phosphate shunt pathway) in the mature erythrocytes. It is considered to be rate limiting for maximal shunt activity. The two most important functions of the pentose shunt in the mature erythrocytes are: (1) the reduction of NADP+ to NADPH via G-6-PD enzyme, and (ii) the production of limited amounts of 5-phosphoribosyl pyrophosphate (PRPP). Both these products contribute to the red cell maintenance and repair. The NADPH remains available to the cell for reductive reactions and other crucial functions mainly having to do with the protection of cell components against oxidation. Thus, this enzyme maintains the reducing power of the cell in the form of NADPH and indeed represents the only source of NADPH available to the erythrocytes (Fertman and Fertman, 1955). PRPP is used by the red cell for the synthesis of adenine nucleotides. The G-6-PD, being the primary enzyme for
supporting directly and/or indirectly the protective mechanisms against oxidant damage, has been studied extensively and deficiencies in this enzyme comprise the most common metabolic defect in the red cell (Beutler, 1971).

The red cell activity G-6-PD (IU/gHb or IU/1x10^{10} RBC) in patients with anaemia of PBM was observed to be significantly low as compared to the activity in normal children (Fig. 32, 33). The results of the present study are in agreement with those obtained by Verjee and Behal (1976) and Fondu et al. (1978c, d) in PBM. The deficiency of G-6-PD could have occurred because of decreased production of apo-protein enzyme molecules due to less availability of proteins. Although several factors which may regulate G-6-PD activity are known, no good model for the combined effect has yet been advanced. It is known for instance that the activity of G-6-PD may be governed by intracellular oxidized glutathione (GSSG) concentration and the rate of oxidation/reduction of glutathione (Jacob and Jandl, 1966). The availability
of glucose-6-phosphate (Rose and O'Connell, 1964), ATP and magnesium (Avigad, 1966) and NADP+ which in turn depends, upon its synthesis from niacin (Rose, 1961) or is formed during reduction of GSSG to GSH by GSSG-R, may also influence the activity of G-6-PD. The availability of some of these factors is expected to be limited in the anaemia of PEM and may also be responsible for diminished G-6-PD activity observed during this syndrome. There was a significant decrease in the reticulocyte count in PEM children as compared to the control. Since, the reticulocytes are rich in this enzyme, the erythrocyte population, relatively poor in reticulocytes in these children, might have some contribution for the diminution of G-6-PD activity.

The dietary rehabilitation of the patients belonging to the first group led to a significant increase in the activity of G-6-PD (Fig. 32, 33). The reanimation of the first group with the diet having high protein contents might have been responsible for the synthesis of apo-protein enzyme molecules leading to increased G-6-PD activity. The reticulocyte count was
low before the start of the dietary treatment but significant reticulocytosis was observed following recovery. The estimation of enzyme in reticulocyte rich blood obviously will result in elevation of enzymic activity. This might be another factor for the observed increase in G-6-PD following dietary realimentation since it is known that the activity of G-6-PD is more in reticulocytes and in young erythrocytes and the amount declines with increasing cell age (Marks, et al, 1958; Bernstein, 1959). Moreover, most of the limiting factors (required for optimal functioning of this enzyme as mentioned previously) which may not be available in PEM, have been replenished by a diet rich in energy, vitamins and minerals. Some of these factors might have also resulted in the increase of G-6-PD activity to near normal level.

The activity of G-6-PD in the red cells was found to be increased significantly after dietary therapy in the second group when it was compared to the initial activity. The increase was much more significant in the second group than in the first group when the
activity was expressed as IU/gHb (Fig. 32). This greater improvement in G-6-PD cannot be explained only on the basis of increased reticulocyte count observed in the second group following realimentation. The activity was slightly elevated as compared to the control, though the increase was not significant. It is obvious that G-6-PD activity improved to a greater extent as compared to the elevation of haemoglobin concentration in the second group. In fact, the dietary treatment of the second group led to the production of iron deficiency anaemia. It may be explained on the basis that the requirements for the synthesis and activity of this enzyme, i.e., proper amino acid pool, niacin (NADP⁺) and others have been provided in the dietary regimen of the second group in contrast to the need of iron for the formation of haemoglobin which was not compensated. Iron-deficiency might have become even more a limiting factor for the generation of haemoglobin in this situation where all other deficiencies have been replenished.

It is, therefore, reasonable to suggest that
the increase in G-6-PD in the second group may be a compensatory mechanism so as to protect the reduced levels of haemoglobin from oxidative damage. There are many reports which indicate that iron-deficiency anaemia is associated with increased activity of G-6-PD in the erythrocytes (Marks, 1958; Vuopio, 1963; MacDougall, 1968; MacDougall et al, 1970) in order to protect the haemoglobin deficient red cells from oxidative damage. It has been suggested that the energy and redox potential of iron deficient red cells may be a compensatory mechanism, secondary to the defective synthesis of haemoglobin and/or stroma (Mac Dougall, 1968). The elevated activity of G-6-PD and increased GSH concentration return to normal after correction of anaemia by iron therapy in these patients. This fact became obvious, though indirectly, from the results of the present study when the activity of G-6-PD and GSH concentration of the two groups after alimentation with two different dietary schedules were compared.

The metabolic activity of the red cell has been reported to be increased in iron-deficiency (Ultmann et al, 1957; Marks, 1958; Mikkila et al, 1960; Sass and
Spear, 1961; Vuopio, 1963) to the presence of young red cell population. This may be true of iron-deficiency anaemia due to blood loss but may not be applicable in case of nutritional iron-deficiency anaemia. However, the situation is quite different in the second group in which the dietary realimentation has led to the development of iron-deficiency anaemia with increased reticulocyte count. Thus the increased activity of G-6-PD may be due in part to the young erythrocyte population and in part to a compensatory response to iron-deficiency.

G-6-PD deficient RBC's undergo oxidative damage and haemolysis when exposed to a number of drugs (Beutler, 1966; Hochstein, 1971). The sensitivity of \( \text{H}_2\text{O}_2 \) derives primarily from the disappearance of GSH. As the GSH concentration falls so does the activity of GSH-Px, the enzyme which is primarily responsible for scavenging the low levels of \( \text{H}_2\text{O}_2 \) within the RBCs (Cohen and Hochstein, 1963; Nicholls, 1972). As the GSH-Px capacity falls off, the intra-cellular levels of \( \text{H}_2\text{O}_2 \) rise (Liebowitz and Cohen, 1968). This increased amount of \( \text{H}_2\text{O}_2 \) cannot be detoxified by the catalase
since its activator, i.e., NADPH is also inadequate. This results in oxidative denaturation of haemoglobin and activation of -SH dependent enzymes leading to Heinz bodies formation and ultimately the removal of damaged RBCs by the reticulo-endothelial system. The diminished red cell survival is probably due to combined effect of metabolic sequelae of this enzyme deficiency.

The erythrocyte is a highly specialized cell densely packed with haemoglobin. It is able to transport oxygen only if its iron is constantly kept in the bivalent state. The red cell is continuously exposed to some degree of oxidative stress from a variety of sources, such as hydrogen peroxide, lipid peroxides/hydroperoxides and other oxidants, including the very oxygen it is destined to transport. It maintains, under normal physiological conditions, its integrity and life span since the erythrocyte is endowed with several protective mechanisms. These are involved in shielding essential enzymes and membrane protein sulfhydryls against inactivation, guarding membrane lipids against peroxidation, and protecting
haemoglobin molecule against irreversible oxidative denaturation. These protective mechanisms (glutathione cascade) depend directly and/or indirectly on the reaction catalysed by the enzyme G-6-PD.

The factors responsible for red cell senescence and destruction have not yet been conclusively identified. It may be said that the 'death' of the normal erythrocyte results from a failure of cellular homeostasis secondary to a diminished capacity of a number of enzymic steps. Alternatively, the red cell destruction may be associated with the progressive decline in the activity of the enzymes responsible for cellular antioxidant defense. The ageing of red cell appears to progressively lose the ability to maintain haemoglobin function which may result from a decline in the efficiency of glutathione cascade. The erythrocyte ageing process is instantaneous in hereditary non-spherocytic haemolytic anaemias and slow in physiological changes due to ageing. The red cell destruction can be explained because of the failure of cell to protect itself against oxidative damage.
The deficiencies of red cell enzymes of glutathione metabolism observed during PEM syndrome are due to the non-availability of various factors and may appropriately be referred to as 'acquired enzymopathies'. In order to understand the importance of acquired enzymopathies, it is worthwhile to consider the factors on which erythrocyte enzyme activity is likely to depend.

i) the amount of apo-protein enzyme molecules synthesized during the development of an erythrocyte;

ii) the primary structure, determining
   a) the normal stability, and
   b) the normal kinetic properties;

iii) the presence of cofactors and coenzymes;

iv) the presence of inhibitors and toxicants; and

v) the destruction of enzyme(?)

Factors (i) and (ii) are regulated by genes but factor (i) may also be influenced by the availability of amino acids whereas factors (iii) and (iv) are
independent of genetic control. About factor (v) very little is known.

The changes in activity of various red cell enzymes have been described in various acquired blood disorders (Valentine et al., 1973; Boivin et al., 1975) such as bone marrow insufficiencies (Heller et al., 1960; Palek et al., 1967; Boivin et al., 1969), leukaemia (Boivin et al., 1970; Zittoun et al., 1970) and dyserythropoiesis (Valentine et al., 1972, 1973). The mechanisms of acquired enzyme defects remain a subject of discussion, and several hypothesis, based on some and/or all of the above mentioned factors, have been put forward to explain them:

i) partial reversion to foetal form of erythropoiesis (Rochant et al., 1972);

ii) the existence of several qualitative and quantitative disturbances in gene expression (Dreyfus et al., 1969; Kahn et al., 1971, 1972; Valentine et al., 1973); and
iii) post-synthetic alterations of some enzymes

A number of above factors have been hypothesized by different workers (Kahn et al, 1971, 1972) to be responsible for decreased activities of various red cell enzymes in acquired blood disorders and is a non-specific phenomenon (Arnold et al, 1974). However, the above mentioned explanations cannot be extended to the anaemia of PEM, since the etiology of this syndrome is nutritional in origin. Rather, in this condition inadequate availability of various ingredients may be the sole factor responsible for the observed enzyme deficiencies. The relationship between these various abnormalities and the existence of a common underlying cause can only be hypothesized. However, the non-availability of specific raw materials for the synthesis of apo-protein enzyme molecules (factor i) and post-synthetic modifications (factors iii) could account for the changes
observed in the erythrocytes, with regards to glutathione metabolism, in the patients with PEM syndrome.

It is known that acquired enzymopathies do not play an important role in mechanism of haemolytic anaemia. The deficiency is usually of moderate severity, similar to the one observed in heterozygous subjects with congenital enzymopathy, who are clinically and haematologically normal (Boivin et al, 1975). However, the occurrence of several moderate deficiencies, in the same cell, especially of the enzymes known to afford protection against oxidative damage, i.e., enzymes of glutathione cascade, such as observed in the anaemia of PEM, would assume an important status. Because of their cumulative effect, they might contribute to the decreased red cell life span, and, as such, be an aggravating factor of anaemia. This is in contrast to the well established hereditary enzyme deficiencies causing haemolytic anaemia. Thus it may be suggested that the situation in PEM is mid-way, i.e., genetic enzymopathies on one extreme and the physiological cellular ageing on the other extreme.
FIG. 4. THE HAEMOGLOBIN LEVELS IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMENS (GROUP-1 AND GROUP-2) ON HAEMOGLOBIN LEVELS IN PEM CASES.
FIG. 5. THE PCV VALUES IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMENS (GROUP-1 AND GROUP-2) ON PCV VALUES IN PEM CASES.
FIG. 6. THE RBC COUNT IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMENS (GROUP-1 AND GROUP-2) ON RBC COUNT IN PEM CASES.
FIG. 7. THE MCV VALUES IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON MCV VALUES IN PEM CASES.
FIG. 8. THE MCH VALUES IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON MCH VALUES IN PEM CASES.
FIG. 9. THE MCHC VALUES IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMENS (GROUP-1 AND GROUP-2) ON MCHC VALUES IN PEM CASES.
FIG. 13. THE RETICULOCYTE COUNT IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP - 1 AND GROUP - 2) ON RETICULOCYTE COUNT IN PEM CASES.
FIG. 14. THE PLASMA IRON LEVELS IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-I AND GROUP-2) ON PLASMA IRON LEVELS IN PEM CASES.
FIG. 15. THE TIBC IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON TIBC IN PEM CASES.
FIG. 16. THE UIBC IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON UIBC IN PEM CASES.
FIG. 17. THE TRANSFERRIN SATURATION IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON TRANSFERRIN SATURATION IN PEM CASES.
FIG. 18. THE TOTAL PLASMA PROTEIN LEVELS IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON TOTAL PLASMA PROTEINS LEVELS IN PEM CASES.
FIG. 19. THE GSH LEVELS IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON GSH LEVELS IN PEM CASES.
FIG. 20. THE GSH LEVELS IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON GSH LEVELS IN PEM CASES.
FIG. 21. THE GSH LEVELS IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON GSH LEVELS IN PEM CASES.
FIG. 22. THE CORRELATION BETWEEN GSH CONCENTRATION AND HAEMOGLOBIN LEVEL IN THE CONTROL GROUP.

\[ Y = 1.4X + 2.3 \]
\[ S_{YX} = \pm 0.42 \]
\[ r = 0.92 \]
\[ P < 0.001 \]
FIG. 23. THE CORRELATION BETWEEN GSH CONCENTRATION AND HAEMOGLOBIN LEVEL IN THE GROUP -1 AFTER TREATMENT.

\[ Y = 0.07 \times + 8.0 \]
\[ S_y. x = \pm 0.97 \]
\[ \gamma = 0.94 \]
\[ P < 0.001 \]
FIG. 24. THE ERYTHROCYTIC GSH-Px ACTIVITY IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON ERYTHROCYTIC GSH-Px ACTIVITY IN PEM CASES.
FIG. 25. THE ERYTHROCYTIC GSH-Px ACTIVITY IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP - 1 AND GROUP - 2) ON ERYTHROCYTIC GSH-Px ACTIVITY IN PEM CASES.
FIG. 26. THE CORRELATION BETWEEN ERYTHROCYTIC GSH-Px ACTIVITY AND PLASMA IRON CONCENTRATION IN THE CONTROL GROUP.

\[ Y = 3.2X - 18.2 \]

\[ SY.X_1 = \pm 3.4 \]

\[ Y = 0.88 \]

\[ P < 0.001 \]

FIG. 26. THE CORRELATION BETWEEN ERYTHROCYTIC GSH-Px ACTIVITY AND PLASMA IRON CONCENTRATION IN THE CONTROL GROUP.
FIG. 27. THE CORRELATION BETWEEN HAEMOGLOBIN LEVEL AND ERYTHROCYTIC GSH-Px ACTIVITY IN THE CONTROL GROUP.
FIG. 28. THE CORRELATION BETWEEN ERYTHROCYTIC GSH-Rc ACTIVITY AND PLASMA IRON CONCENTRATION IN THE PEM GROUP.

- ON ADMISSION
- TWO WEEKS AFTER TREATMENT

$Y = 2.2X + 4.7$
$S_{Y,X} = \pm 2.8$
$\gamma = 0.90$
$P < 0.001$

$Y = 4.6X - 55.2$
$S_{Y,X} = \pm 2.7$
$\gamma = 0.96$
$P < 0.001$
FIG. 29. THE CORRELATION BETWEEN HAEMOGLOBIN LEVEL AND ERYTHROCYTIC GSH-PX ACTIVITY IN THE PEM GROUP-1.

--- ON ADMISSION
--- TWO WEEKS AFTER TREATMENT

\[
Y = 2.6 \times 0 + 4.4 \\
\text{SY.} \times \pm 1.8 \\
Y = 0.97 \\
P < 0.001
\]

\[
Y = 4.9 \times 14.5 \\
\text{SY.} \times \pm 1.2 \\
r = 0.99 \\
P < 0.001
\]
FIG. 30. THE ERYTHROCYTIC GSSG-R ACTIVITY IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMENS (GROUP-1 AND GROUP-2) ON ERYTHROCYTIC GSSG-R ACTIVITY IN PEM CASES.
FIG. 31. THE ERYTHROCYTIC GSSG-R ACTIVITY IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMENS (GROUP-1 AND GROUP-2) ON ERYTHROCYTIC GSSG-R ACTIVITY IN PEM CASES.
FIG. 32. THE ERYTHROCYTIC G-6-PD ACTIVITY IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMENS (GROUP 1 AND GROUP 2) ON ERYTHROCYTIC G-6-PD ACTIVITY IN PEM CASES.
FIG. 33. THE ERYTHROCYTIC G-6-PD ACTIVITY IN THE NORMAL CONTROLS AND THE PEM CHILDREN AS WELL AS THE EFFECT OF TWO DIETARY REGIMEN (GROUP-1 AND GROUP-2) ON ERYTHROCYTIC G-6-PD ACTIVITY IN PEM CASES.