NUTRITIONAL ANEMIAS:

SPECTRUM AND PERSPECTIVES WITH RELEVANCE TO MALAYSIA

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PREFACE

Recent local data on the anemia problem are scarce. Data from studies elsewhere are also not easy to obtain as these have been reported in many different kinds of of journals, dispersed in the various libraries. Recent text books on the subject are also difficult to obtain. It is thus felt that a thorough review of the subject could assist an intending investigator into the field. Such a review should cover a wide spectrum of the subject. It should include recent reports so that current knowledge of the problem may be presented. Since locally available data have not been thoroughly compiled, the review should attempt to present the status of the problem in the country.

It is hoped that this review will meet some of the objectives outlined. Through a total of 324 references, out of which 52 are reports of local investigators, it is hoped that the reader will have a clear understanding of the work done in the country, in the overall perspectives of recent knowledge on the problem of nutritional anemia. It is hoped that it can provide the appropriate references for further reading and pursuance.

I would like to take this opportunity to record my sincere gratitude to Associate Professor Abdul Kader Md. Hussain, Consultant Perinatologist/ Neonatologist of the Perinatal Unit, Department of Pediatrics, Universiti Kebangsaan Malaysia, Kuala Lumpur, and Dr. Khalid Hassan, Head of the Division of Hematology of the Institute for Medical Research. Inspite of their tight schedule and heavy committments, they have read through the draft of this review very thoroughly and made some very pertinent comments and suggestions. They have been most encouraging and supportive of my effort to produce this work. I would also like to thank Dr. G.F. De Witt, Director of this Institute, and Dr. Y.H. Chong, Head of the Division of Human Nutrition, for their support and permission to publish this review. I am also thankful to Professor Hamid Arshat of the Specialist and Reproductive Research Centre of the National Population and Family Development Board, Malaysia, for accepting the work to be published as a monograph of the Centre, and Encik Jaffar Ali, also of the said Research Centre, for his efforts in coordinating the publication of this review.

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1 INTRODUCTION

Nutritional anemia is an important problem affecting large population groups all over the world, especially in developing countries (WHO, 1975). Continuing efforts have been mounted towards understanding and characterising this disorder. The World Health Organization, since 1949, has been interested in the problem (Patwardham, 1966) and has since initiated and coordinated various studies and programs in different part of the world. As a result of all the research activities of individuals, national efforts and international collaboration, the understanding of this global problem has increased tremendously. There is now a common general understanding of the etiology of the disorder; the body's requirements for the hemopoietic nutrients are better defined; there are now improved techniques and methodologies to map out the prevalence and extent of the problem; the deleterious effects of nutritional anemias are now better understood and appreciated; and there are now generally accepted intervention programs for the control or alleviation of the problem.

Research in nutritional anemias has been active in Malaysia in the 50s and 60s. Various efforts were made to characterise the disorder as it occurred in the country. Prevalence of the disorder were studied in various communities. Continued efforts in the 70s and recent years have shown that nutritional anemia is still a major problem here, as it is in many parts of the world. It is therefore necessary to continue to keep a vigilance on the problem. There has to be a continuous surveilance on the extent of the problem. This will enable appropriate intervention measures to be implemented.

This review will attempt to present as "complete" a picture of the nutritional anemia problem as possible. A clear definition of what nutritional anemias are and the hemopoietic nutrients involved will first be given. The various factors involved in and/or related to a balance of these nutrients in the "normal" individual will then be discussed, encompassing such aspects as how these nutrients are stored in the body, through what channels the body loses them, the efficiency with which the body absorbs them from the diet, and the daily requirements for these nutrients. Following this, factors that could bring about an imbalance, i.e. resulting deficiency, will be discussed. The methods of diagnosis and measurement of such deficiencies will then be elaborated. The review will also discuss current understandings on the deleterious effects these deficiencies could have on the body and health. Finally, it will consider the intervention measures that may be taken to control or alleviate such deficiencies in the community. In the areas just outlined, wherever appropriate, recent findings and understandings will be discussed. In those areas where Malaysian studies have been reported, their data will be reviewed in some detail. It is hoped that in this way, the review can provide a clear understanding of the work done in the

country in the overall perspectives of recent knowledge on the problem of nutritional anemias. This review will however in no way be comprehensive; it is not meant to be so. It cannot go into details of specific areas of the problem, for which numerous books and reviews are available. It however hopes to provide the reader with a fairly complete idea of the whole specturm of the problem and provide the appropriate references for further reading and pursuance.

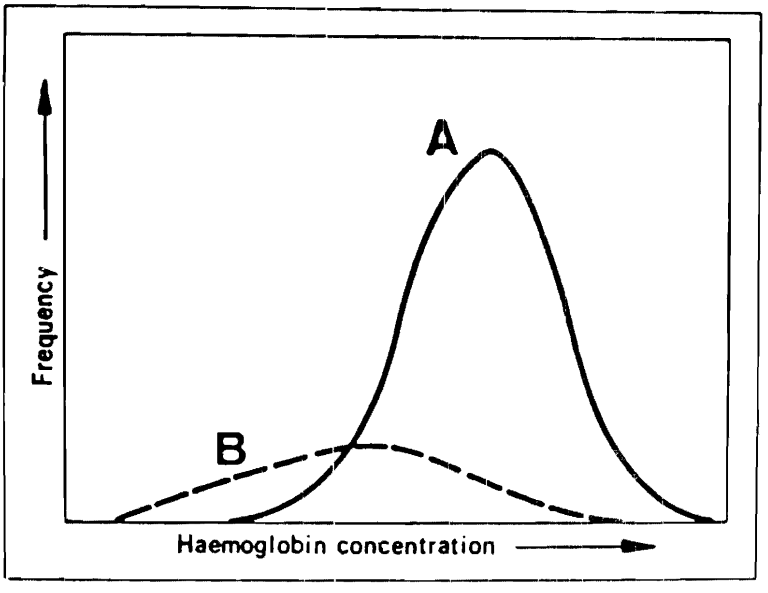
2 DEFINITIONS

As defined by the World Health Organization (WHO, 1968), nutritional anemia is "a condition in which the hemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency". For practical purposes of characterization of anemia, a concentration of hemoglobin has been defined below which anemia is said to exist. It has been realised that this was an over simplification (WHO, 1972), but it is thought to be the only one available for dealing with individuals (Layrisse, Roche and Baker, 1976).

The difficulty of defining "normal hemoglobin concentration" is well recognized. A WHO Expert Group on nutritional anemias met in late 1971 to review the definition (WHO, 1972). The Group noted that there is a homeostatic mechanism that sets the hemoglobin level in each individual. Whereas it is not known whether this is the optimum level for health, it is accepted as "normal" for the individual. The Group suggested that the distribution of such normal values in the population could be derived from a representative sample of healthy persons in whom the presence of nutritional deficiencies has been excluded by specific laboratory assessment or by the prior administration of hematinics. It was felt that this distribution of normal values is likely to be the same throughout the world when allowance is made for such factors as age, sex, pregnancy, and altitude. The distribution of individual hemoglobin concentation of an adequately nourished population so obtained may be represented by curve A in Figure 1 (from WHO, 1972). The distribution of hemoglobin for the anemic persons in the community may be represented by curve B in the same Figure, which is seen to be skewed to the left, but overlaps to some extent the curve for subjects with normal hemoglobin. Thus there is no value for hemoglobin concentration that will separate anemic from normal subjects with certainty. It was therefore recommended that in studies of nutritional anemias of populations, it would be preferable to characterize their status by frequency distributions of hemoglobin concentration rather than by relating it to a single arbitrary value. From such information, the probablity that anemia is present at a given hemoglobin concentration may be assessed.

Baker and Demaeyer (1979) have re-defined nutritional anemia as "a condition in which the hemoglobin concentration is below the level that is normal, for a given individual, due to deficiency of one or more of the

Figure 1. Theoretical Frequency Distribution Curves for Haemoglobin Concentration in (A) Subjects with Normal Haemoglobin Levels and (B) Subjects with Anemia.



Source: WHO 1972

nutrients required for hemopoiesis, and, conversely, as a condition in which the hemoglobin concentration can be raised by increasing the amount of nutrient(s) absorbed". It has thus been proposed that by measuring the hemoglobin concentration or hematocrit of a population, supplementing the population with hemopoietic nutrients and then repeating the estimations of hemoglobin or hematocrit, it would be possible to define the responders in the population and hence, by the above definition, determine the individuals who were anemic (see Section 6.1.3.3. on Laboratory Diagnosis : therapeutic trials). It would then be possible to calculate the odds of an individual with a given hemoglobin or hematocrit value being anemic. However, from the point of view of public health practice, Baker and Demaeyer (1979) feel that in order to have some simple way of defining the prevalence and severity of anemia in a community, it is desirable to combine the concepts of frequency distribution and an arbitrary standard.

3 HEMOPOIETIC NUTRIENTS

The main hemopoietic nutrients are iron, folic acid and vitamin B_{12}. The role of proteins in nutritional anemias is felt to be less crucial, except amongst those with protein deficiency (Thanangkul, 1980). Less important nutrients include cobalt, copper, ascorbic acid, pyridoxine and vitamin E. Although there is experimental evidence that these latter nutrients may be necessary for hemopoiesis, anemia due to deficiencies of these nutrients are presumed to be so rare that, from the public health point of view, they may be ignored (Baker and Demaeyer, 1979). It is now clear that iron deficiency is by far the commonest cause of anemia, followed by folate deficiency. Vitamin B_{12} deficiency is thought to be a less important cause. Some basic aspects of each of these three major hemopoietic nutrients will be discussed, including their chemistry and function.

3.1. IRON

The importance of iron to the well-being and progress of mankind appeared to have been recognized long before medical practice became firmly established. Fascinating accounts of the history of iron in medicine have been given by Diamond (1970) and Fairbanks, Fahey and Beutler (1971). According to these accounts, the use of iron is said to go back to the earliest days of mythology and is based on many legends. The Greek word for iron is "sideros". The Latin "sidereus" means a constellation or a star, suggesting that iron might have been believed to be a special gift from the heavens. The Greeks believed it to be sent by Mars, the Warrior God, and his name was used by the ancients as a synonym for iron.

Iron, as it is known today, is element number 26 in the periodic table and has an atomic weight of 55.85. It is the fourth most abundant element and the second most abundant metal in the earth's crust (Bothwell *et al.* 1979). A detailed discussion of the chemistry of iron has been given by Spiro and Saltman (1974). It is an invariable essential constituent of all living organisms (Fairbanks, 1978). Because it can readily accept or donate electrons, the iron molecule serves as a catalyst in biologic processes requiring oxidation and reduction. The incorporation of iron into a variety of molecules and the complexing of it to various proteins confer specificity and enhance this metabolic activity (Pearson and Robinson. 1976). Iron participates in diverse living processes, ranging from the activation of oxygen, nitrogen and hydrogen to the control of electron flow through numerous bioenergetic pathways (Bothwell *et al.* 1979).

3.2 FOLIC ACID

The series of events leading to the discovery of folic acid has been outlined by Chanarin (1979) and Malin (1975). A series of observations in the

1930s and 40s were said to have indicated the presence of a hitherto unidentified anti-anemic agent or growth factor required by humans, monkeys, chicks and micro-organisms. These observations were followed by isolation of a relatively pure sample of the essential factor. Its chemical structure was subsequently determined by the identification of its degradation products and the final proof of the structure of folic acid was obtained by its chemical synthesis.

Folic acid is a compound containing a pteridine linked to p-aminobenzoic and L-glutamic acid, as shown in the structural formula given in Figure 2. The term "pteroylglutamic acid" was assigned because of the presence of pteridine and glutamic acid in the molecule. The name "folic acid", propos-

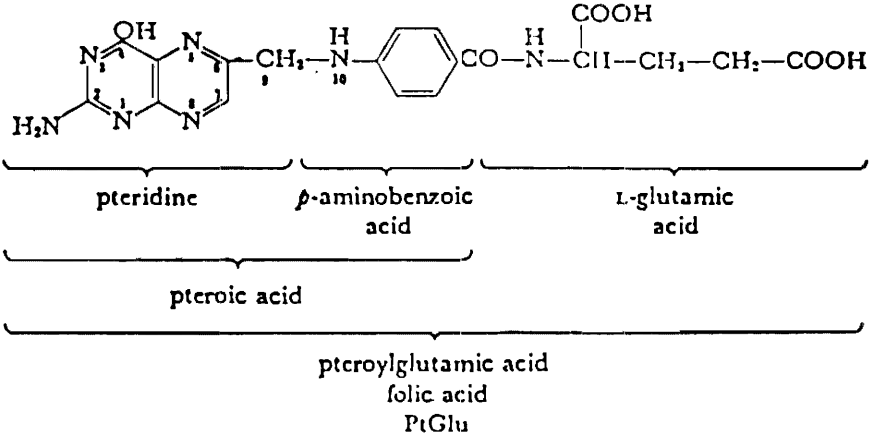


Figure 2. Folic Acid (Pteroylglutamic Acid)

ed on the basis of its prevalence in leafy materials ("folium" is the Latin word for leaf) is generally used. The Joint Nomenclature Committee of the American Institute of Nutrition and the Society of Biological Chemists had however proposed the name "folacin" (Stokstad and Koch, 1967). Folic acid is known to exist in plant and animal tissues largely in the form of conjugates containing more than one glutamic acid, the pteroylpolyglutamates.

It is now known that folic acid is a water-soluble compound within the vitamin B complex, and has physiological and biochemical importance in the metabolism of many organisms, especially the higher animals and man. Folic acid itself, however, is not biochemically active but becomes so after it has undergone reduction in the pteridine ring. This can be brought about enzymatically or chemically to produce dihydrofolic acid or tetrahydrofolic

acid. Like many other vitamins, folic acid is a precursor of a coenzyme involved in enzymatic reactions. The function of folate coenzymes is primarily concerned with the transfer of one-carbon units at the oxidation levels of formate, formaldehyde and methanol and in transforming these from one oxidation state to another. The principal metabolically active formyl derivatives of tetrahydrofolic acid are: N^{10}]-Formyltetrahydrofolic acid, N^5 -Formyltetrahydrofolic acid, N^5 , N^{10}-Methenyltetrahydrofolic acid, N^5 -Formininotetrahydrofolic acid, N^5 ,N^{10}-Methylenetetrahydrofolic acid and N^5 -methyltetrahydrofolic acid. These participate in such important reactions as purine and pyrimidine synthesis, and metabolism of various amino acids, such as the well-known methionine methyl group biosynthesis. Further details of the chemistry and metabolism of folic acid are given in e.g. Stokstad and Koch (1967), Chanarin (1979), Blakley (1969), Malin (1975) and Scott and Weir (1976).

3.3 VITAMIN B_{12}

Smith (1965) and Pratt(1972) provided interesting accounts of the discovery of vitamin B_{12}. The history of B_{12} was said to have commenced with the first description of pernicious anemia in 1821. For the next one hundred years, there was a slow but steady increase in the understanding of the signs of the disease and the methods of diagnosis, but a total lack of any advance in the treatment of the disorder. Then in the 1920's, investigators had observed that there were favourable hematological responses in patients fed with diets rich in liver. It was thus thought that the liver must contain something, then called the "liver factor" or the "anti-pernicious anemia factor" that brought about the striking effects observed. Attempts were then made to extract and isolate this factor from liver. At around the same time, other investigators had observed that the atrophied gastric glands of pernicious anemic patients might be failing to secrete some essential kind of digestive juice which was later called "intrinsic factor". This was thought to act upon something present in certain foods, called the "extrinsic factor". The race to isolate the "liver factor" was said to be very close : isolation of crystalline vitamin B_{12} was reported independently by two industrial laboratories within the space of a few weeks, namely Merck in America and Glaxo Laboratories in England, in the late 1940s.

The vitamin B_{12} molecule (described in Chanarin, 1979 and Rottenbreg and Cotter, 1978) consists of two major portions (see Fig. 3). One is the corrin ring which bears a close resemblance to a porphyrin, consisting of four pyrrole-type units coupled directly to each other with the inner nitrogen atom of each pyrrole coordinated with a single atom of cobalt. The other portion of the molecule is a nucleotide 5,6-dimethyl benzimidazole which is set nearly at right angles to the corrin portion and coupled directly to the cobalt atom. An

ester linkage from the phosphate group of the nucleotide to the propionic acid group of the D-ring of the corrin nucleus adds further stability to the nucleus. Cyanide, which lies above the planar ring and is attached to the cobalt atom, is actually an artifact of isolation and is replaced by the deoxadenosine and a methyl group in the active coenzyme forms of the vitamin.

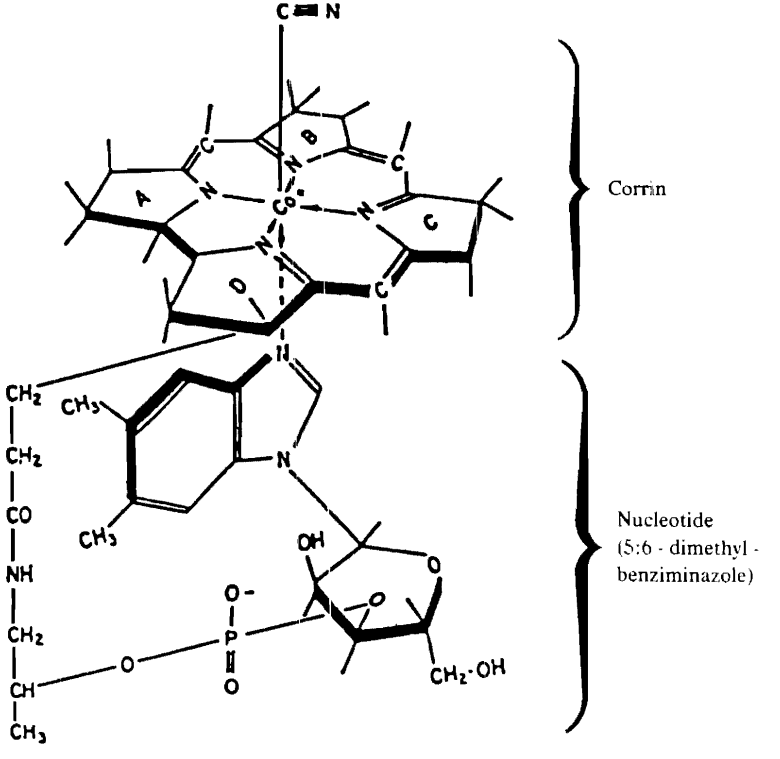


Figure 3. Vitamin B_{12} (Cyanocobalamin)

Vitamin B_{12} or cyanocobalamin crystallizes as dark, red, needle-like birefringent hygroscopic crystals containing 10-12 % moisture. It is fairly soluble in water. Its molecular formula is $C_{63}H_{88}O_{14}H_{14}PCO$, with a molecular weight of 1355. Other chemical and physical properties of the vitamin are described by Smith (1965), Moore and Folkers (1968) and Chanarin (1979).

Like other vitamins, B_{12} is required by humans and other animals in small amounts to perform (with or without further structural modification) some catalytic role. It is the only vitamin which contains a metal, i.e. a co-ordination compound, and is also the largest of the vitamins and apparently

the only one which requires a special mechanism for absorption into the body from the gut. It is also known that the vitamin can only be synthesized by microorganisms (certain bacteria, moulds, blue-green, brown or red algae but not green algae) (Pratt, 1972). The enzymic functions of vitamin B_{12} have been described in numerous texts and reviews, such as Smith (1965), Barker (1968), Chanarin (1979), Silber and Moldow (1970) and Pratt (1972). There are at least three types of reactions in which vitamin B_{12} plays an important role. It plays a central role in single carbon unit metabolism, such as methylation of homocysteine to form methionine. Here it acts as a methyl group carrier for the enzyme methyltetrahydrofolate-homocysteine methyltransferase which catalyzes the above mentioned conversion N^5-methylterahydrofolate is the methyl donor and tetrahydrofolate is also product of this reaction. Since this is a primary pathway for the metabolism of N^5-methyltetrahydrofolate, cobalamin deficiency actually impairs the generation of tetrahydrofolate. This is the fundamental biochemical link which interrelates vitamin B_{12} and folic acid metabolism (Rottenberg and Cotter, 1978). Besides this role, vitamin B_{12} is also important in isomerization reactions and oxidation-reduction reactions.

There is a close metabolic interaction between vitamin B_{12} and folic acid. Nixon and Bertino (1970) and Das and Herbert (1976) reviewed in some detail the metabolic roles and interrelation of these two vitamins.

4 NUTRIENT BALANCE

The healthy, adequately nourished individual is normally in a state of nutritional balance in which the amount of any given nutrient absorbed from the diet is equal to the amount of nutrient broken down in metabolic processes and/or the amount lost from the body. This balance can be disturbed by one or more of a variety of factors which lead to a relative or absolute deficiency of the nutrient - in this context, iron, folate and vitamin B_{12}. These interrelated factors are: (1) increased losses, (2) increased requirements, (3) decreased uptake in the diet, (4) decreased absorption, and (5) decreased utilization (Baker and DeMaeyer, 1979). In order to appreciate fully the resulting deficiencies in the event of an imbalance, it would be necessary first to examine the factors involved in bringing about this nutrient balance. Since body stores of these nutrients will greatly influence the other factors, this will be first discussed, followed by a description of the possible avenues of loss of these nutrients from the body. From the latter, daily nutrient requirements can be calculated. After considering the efficiency of absorption of these nutrients from foods, recommended dietary requirements could be derived.

4.1 IRON BALANCE

4.1.1 Body Iron

For convenience, body iron has been divided into that which is "functional compounds" essential for normal function and those present as "storage or reserve iron" (see Table 1). In the past decade, extensive studies have been undertaken to understand the molecular structure of these important iron-containing molecules and relating this to their functions. A vast body of knowledge is now available in various standard textbooks and recent reviews, e.g. Fairbanks, Fahey and Beutler (1971), Kief (1975), and Worwood (1982). According to the concentrations given in Table 1, total body iron of normal adult males can be calculated to be 3-4 g; women have lesser amounts and the distribution differs slightly from that in men. The distribution of iron in the infants and its increment during growth has been tabulated by Burman (1974).

As can be seen in Table 1, the major portion (approximately 60-70%) of the body iron is found in the iron-porphyrin complex hemoglobin. Its concentration in the red blood cells has been estimated to be about 1 gram per kilogram (Finch and Huebers, 1982). Most attention has been directed towards this complex, found exclusively in the erythrocytes, since iron status has often been measured through a determination of its concentration in the blood. Hemoglobin is a protein with a molecular weight of 64,456 and contains 0.34% iron by weight (Fairbanks, 1978). It is responsible for the transport and exchange of oxygen and carbon dioxide in the blood. Oxygen is taken up in the lungs and transported in this pigment by the circulatory blood to the tissues, where hemoglobin releases the oxygen and takes over some carbon dioxide transport in the venous circulation.

Table 1. Normal Concentrations of Body Iron in Adults

Type of iron	Concentration			
	men		women	
	μg/kg body wt	(%)	μg/kg body wt	(%)
Functional compounds				
Hemoglobin	31	62.0	28	73.7
Others (myoglobin, heme enzymes and non-heme enzymes, transferrin)	6	12.0	5	13.2
Storage complexes (ferritin and hemosiderin)	13	26.0	5	13.2
Total	50		38	

(retabulated from: Finch and Huebers, 1982)

The next largest fraction of the functional iron compounds is myoglobin. It is closely related to hemoglobin structurally but is monomeric containing a single heme group, whereas the latter is tetrameric. Thus the molecular weight of myoglobin is approximately one fourth that of hemoglobin. It occurs in striated muscles (skeletal muscle and myocardium), where it may serve as an oxygen reservior to be used under circumstances of extreme oxygen deprivation. In man, the myoglobin content of muscle is very small (about 0.01 g/kg of muscle) and it does not appear to have a major physiologic role or contribute in a major way to iron metabolism (Fairbanks, 1978).

The remainder of the essential tissue iron or functional iron include a large number of enzymes and co-enzymes classified as : (a) heme enzymes, e.g. cytochromes a, b, c, c_1 , a_2, cytochrome c oxidase, lipoxidase, catalase, trytophan pyrrolase, homogentisic oxidase and peroxidases: (b) iron-flavoproteins, e.g. cytochrome c reductase, succinate dehydrogenase, acyl-CoA dehydrogenase, NADH dehydrogenase and xanthine oxidase; (c) enzymes requiring iron as a cofactor, e.g. aconitase and succinate dehydrogenase (Fairbanks, Fahey and Beutler, 1971). The amount of iron in this compartment is the smallest in the body. It is nevertheless a very active compartment. These iron enzymes are involved in the metabolism of most of the cells in the body and are probably indespensable to life. A well studied example is the ubiquitous cytochromes which function as reversible acceptor-donors of electrons. Some of the iron enzymes and cytochromes are very sensitive to changes in total body iron and exhibit decreased activity very early in the course of iron depletion (Fairbanks, 1978).

A very small percentage (about 0.08%) of the body iron is present in the plasma protein, transferrin (or siderophilin). It appears to be a heavily hydrated, somewhat elongated single polypeptide chain with a molecular weight of about 75,000. Transferrin is the major iron transport protein in the body. It transports iron from storage sites to developing red cell precursors in the bone marrow. It appears to carry out this function by having two binding sites for trivalent iron. Thus, it can exist as monoferric, diferric or apoferric transferrin containing one, two or no iron atoms, respectively. Normally, circulating transferrin is only about 30% saturated with iron (Aisen, 1982). This percentage of transferrin saturation decreases during the iron deficient erythropoiesis stage of iron deficiency, when there is a diminished supply of iron to the erythroid precursors. It is one of the most frequently used indicators in the diagnosis of iron deficiency anemia (discussed in 6.1.2). A more detailed discussion of transferrin is given in Morgan (1974).

Some authors have discussed the concept of a "labile iron pool" in the body. Unlike other iron compartments, this "pool" is a functional, hypothetical concept, derived from iron kinetic studies. It has not been defin-

ed in terms of anatomic location, histochemical characteristics or biochemical properties. Fairbanks, Fahey and Beutler (1971) have discussed some problems with this attractive model of the "labile iron pool".

Next to hemoglobin, the largest iron compartment in the body is the storage iron, most of which is held as ferritin and hemosiderin. The ferritin molecule (described by Harrison *et al,* 1974; Crichton, 1975) consists of two components : a multisubunit protein, apoferritin and a ferric oxyhydroxide micelle. The protein forms a spherical shell of 12-12.5 nm which has a central cavity of 7 nm in diameter: the micellar iron core lies in this cavity. Apoferritin has a molecular weight of 450,000 and consists of 24 identical polyeptide chains of molecular weight 18,500. The iron content of ferritin may vary from zero to a maximum of 4.300 atoms of iron per molecule. Thus, when in maximum capacity, 27% of the dry weight of the molecule is iron. Ferritin is found principally in the liver, spleen and bone marrow, although it is probably also present in most other human tissues. In most normal adults serum ferritin concentrations lie within the range of 15-300 ug/dl but concentrations are dependent on both age and sex (Worwood, 1982). In man, it serves a number of functions, the most important of which is to act as an iron depot which can provide iron for the synthesis of iron-containing proteins (such as those discussed above). It has been shown that serum ferritin levels are directly proportional to body iron stores. The measurement of serum ferritin level has been regarded as the most sensitive parameter of iron status since storage iron depletion is the earliest stage of iron deficiency (discussed in detail in 6.1.1). Ferritin is also of importance, in conjunction with hemosiderin, in the protection of cells against the toxic effects of free ferric iron (Worwood, 1982).

Hemosiderin is the other major storage form of iron. It appears to be a degraded form of ferritin in which the molecules have lost part of their protein shell and have aggregated (Worwood, 1982). In contrast to ferritin, hemosiderin is water insoluble and is thus easily seen microscopically in bone marrow smears or tissue sections stained by the Perls method. It contains about 25-30% iron by weight (Fairbanks, 1978). The ratio of ferritin to hemosiderin in the liver varies according to the total amount of iron present: at lower iron concentrations, ferritin predominates, whereas at higher levels, most iron exists as hemosiderin (Finch and Huebers, 1982).

Total iron stores (ferrition and hemosiderin) are at their lowest level between 12 and 60 months of age; during childhood and adolescence they increase slowly and reach adult levels in males around 20 years of age (Layrisse, Roche and Baker, 1976).

4.1.2 Iron Losses

In males and non-menstruating females, the major routes of the basal physiological losses or obligatory losses are via the gastrointestinal tract (70%), the skin (20%) and the urine (10%) (Burman, 1982). These losses have been estimated to be 14 ug/kg body weight/day (WHO, 1970). It is however known that with higher iron stores, daily losses can be increased by about two-fold; in iron deficiency states, it can be reduced to about half the usual losses (Green *et al,* 1968). Little is known of the physiological losses in infants and children; they are usually assumed to be proportional to that of-the adults (Bothwell *et al,* 1979).

In women of child-bearing age, menstrual losses of iron must be added to the basal losses mentioned above. In a study of 476 women in Sweden, Hallberg *et al* (1966) reported a mean blood loss of about 38 ml in healthy subjects with normal menstrual losses. Examining reports of measurements of such losses from various countries, Cole, Billewicz and Thomson (1971) and Golther (1975) noted a wide range of menstrual losses and that there was a positive skew in the distribution curve; mean values of around 30 ml were common.The pattern however appears to be similar in the population groups studied and the amount of blood lost each month was said to be fairly constant (Baker and DeMaeyer, 1979).

It is commonly held that one of the causes for the high prevalence of iron deficiency in the tropics is that there is excessive iron loss in sweat. The elaborate long-term study of Americans, Venezuelans and Africans reported by Green *at al* (1968) however has shown that the subjects studied were losing iron at a similar rate of about 10% per annum, irrespective of environmental tempreature or the amount of sweating. This study also shows that the main loss of iron from the skin is in the shed epithelium indicating that sweating is not a factor in producing the high prevalence of iron deficiency in tropical countires.

Studies cited by Roche and Layrisse (1966) and Baker and DeMaeyer (1979) have implicated parasitic infection as the major cause of anemia as early as at the end of the 19th century. Of the various parasites, hookworm infestation is said to be rivalled only by malaria as a common infective cause of severe anemia in the tropics (Fleming, 1982). Thus, the importance of iron losses due of parasites in the pathogenesis of tropical iron deficiency anemia was well appreciated. Numerous studies have been carried out to determine the extent of this loss. In a fairly recent study, Layrisse and Roche (1964) had demonstrated that with hookworm egg counts of over 2000/g faeces, there was a significant correlation between the severity of the infestation and the degree of anemia. In a later report (Roche and Layrisse, 1966) these in-

vestigators reported that blood loss was observed to vary from 2 to 3 ml per day in lightly infested subjects to around 100 ml in heavy infestations. Blood loss per worm due to these parasites was reported to be 0.03 ml per day for *Necator americanus,* 0.05 for *Ancylostoma caninum* and 0.15 for *Ancylostoma duodenale.* Nett daily iron loss with a worm load represented by an egg count of 1000 egg/g faeces was reported to be about 0.7 mg for *N. americanus* infestation and about 1.2mg for *A. duodenale.* Considering that hookworm infestation has been estimated to be present in more than 600 million people living in Central and South America, Africa, Asia andOceania (Layrisse, Roche and Baker, 1976), these findings are of great significance.

Trichuris trichiura is another parasite that sucks blood from the intestinal mucosa. Infestation occurs mainly among children. Blood loss due to these parasites could amount to about 5 µl (0.005ml) per worm (Layrisse *et* al, 1967). Unlike in hookworm infestation, there is little opportunity for the reasbsorption of iron, so that with a heavy worm load, a significant degree of iron loss may result. It is thought to cause profound iron deficiency anemia in several areas (Fleming, 1982).

There has also been some indication that chronic blood loss can occur with *Schistosoma hematobium* and *S. mansoni* infection, and this could be of significance where schistosomiasis is a major problem (Fleming, 1982).

4.1.3 Iron Requirements

To maintain nutritional balance, the daily intake of iron must replenish the amount lost from the body as discussed above, and in addition must meet all the requirements needed under all physiological conditions. Daily requirements of iron as recommended by WHO (1970 and 1972) and retabulated by Baker and DeMaeyer (1979) are given in Table 2. It is to be

Table 2. Daily Requirements of Iron

Age group	Daily requirements of iron* (mg)
Infants, 5 - 12 months	0.7
Children, 1 - 12 years	1.0
Boys, 13 - 16 years	1.8
Girls, 13 - 16 years	2.4
Menstruating women	2.8
Men	0.9
Pregnancy, lst half	0.8
Pregancy, 2nd half	3.0
Lactation	2.4

* The amount that must be absorbed to maintain homeostasis.
Source: Baker and DeMaeyer (1979).

noted that the amounts tabulated refer to the quantities effectively absorbed from the food and made available to the body to maintain homeostasis. Quantities of iron recommended to be present in foods will be considered later, after discussing iron absorption from food in the next section. A brief description of the derivation of these requirements is presented in the following paragraphs.

If the daily basal physiological losses in adult males are taken as 14 ug/kg (as discussed above), then in order to maintain homeostasis in a 65 kg man, 0.9 mg iron must be absorbed from the food and become effectively available to the individual. In women of reproductive age, extra iron must be made available to cover the amount lost by menstruation in addition to the basal physiological loss, also taken to be 14 ug/kg body weight per day, or 0.8 mg in a 55 kg woman. In considering the menstrual loss, the data of Hallberg *et al* (1966) is useful. It was found that 95% of the women suffered a menstrual loss of 2.0 mg iron per day. Hence WHO (1970) has estimated the daily iron requirement for menstruating women to be 2.8 mg (0.8 mg + 2.0 mg), to ensure that 95% of the population will be in positive iron balance.

While pregnancy affords a temporary relief from the menstrual iron drain, the overall amount of iron required is always greater than that of 9 months of non-pregnant life. Thus in pregnant women, iron is needed to meet:

(a) the obligatory basal losses which are assumed to be similar to those in the non-pregnant state (about 220 mg (0.8 mg x 280 days) for the whole period of gestation);

(b) the increase in maternal red-cell mass (about 500 mg); and

(c) the requirement of the fetus (about 290 mg) and placenta (about 25 mg).

The total requirements for the whole pregnancy is therefore about 1000 mg (WHO, 1970).

It is however well established that the demand for iron is not evenly spread throughout the pregnancy. Requirements are probably high during the later half of pregnancy, since iron is transferred to the growing fetus and placental structures in progressively increasing amounts. Assuming that the red cell mass begins to increase at the mid-points of pregancy and that the increase is linear to the end of pregnancy, then the maximal daily iron need for this purpose could be derived by dividing the total increase in red cell mass iron (500 mg) by the remaining number of days of gestation (140), i.e. 3.6 mg per day. At the same time, the daily needs of the fetus would be expected to increase from almost zero at the mid-point of pregnancy to about 4.0 mg per day at the end of the pregnancy. On this basis, WHO (1970) recommended

the iron requirements during the first half of pregnancy to be 0.8 mg per day (to take care of the basal physiological loss); at mid-pregnancy, 4.4 mg per day (0.8 mg + 3.6 mg) is required (to include the increase in red cell mass) and as high as 8.4 mg per day (0.8 mg + 3.6 mg + 4.0 mg) at the end of the pregnancy (after adding in the iron requirement for the fetus). Bothwell *et al* (1979) had recommended an iron requirement (in mg/day) of 0.8 for the first trimester, 4.0 in the second, and 6.0 in the third trimester.

These requirements would necessarily depend on the existing iron stores of the woman at the time of entering into pregnancy. If some iron reserves are available (say 500 mg), then the iron needed to cover the increase in red cell mass (500 mg) can be ignored in the calculation of iron required during the second half of pregnancy. This assumes that the red cell iron is retained in the body and will be returned to stores at the end of pregnancy while the iron lost through blood loss at and after parturition may be replaced during lactation. The nett daily requirement of iron during the second half of pregnancy (WHO, 1970), as indicated in Table 2, would then be about 425 mg (basal loss 100 mg; fetus , 290 mg; placenta, 25 mg) divided by the remaining number of days of gestation, i.e. 425/140 = 3.0 mg. Since a considerable number of women in developing countries are expected to have poor or no iron reserves, Baker and Demaeyer (1979) felt that there is a strong case for the routine administation of iron supplements to all pregnant women in the tropics.

During lactation there are usually no menstrual losses, but there is additional iron loss through lactation. Baker and DeMaeyer (1979) felt that the daily secretion of iron in breast milk probably does not exceed 0.2 mg although Bothwell *et al*, (1979) gave a higher estimate of 0.5 mg - 1.0 mg daily. In addition, during this time, the losses occasioned by hemorrhage at delivery (about 250 mg) must be made up. Taking these into consideration, WHO (1970) has recommended a daily requirement of 2.4 mg for this period (see Table 2). The recommended daily requirement is made on the assumption that there is a negligible iron "debt" incurred during pregnancy and that there is a lactation period of 6 months.

During the first 4 months of life of a normal breast-fed infant there is little or no increase in body iron (WHO, 1970). Iron is made available to the growing tissues by a process of redistribution - hence a resulting decrease of hemoglobin mass. If iron stores were normal at birth, iron absorption probably only needs to meet the basal losses. It is assumed that the iron content of breast milk (about 0.2 mg/day) is adequate to meet the obligatory losses in the breast-fed infant (Baker and DeMaeyer, 1979). However, from about the 4th or 5th month of life, total body iron increases. Taking the prediction model used by Hawkins (1964) that the body iron content at 1 year of age is about 400 mg and that the body iron content at the end of the 4th month is about 290 mg, then the iron requirement for the remaining 8 months of in-

fancy can be estimated to be about 0.5 mg per day (400 - 290mg/240 days). This, together with the basal losses (0.2 mg daily) puts the requirement at about 0.7 mg per day (see Table 2).

With the continued growth of the young child, total body iron increases as do basal losses. Until adolescence is reached, the requirement has been recommended (WHO, 1972) to be 1.0 mg per day in both sexes. At the growth spurt of the adolescent boys (13-16 years) the increase in body mass and hemoglobin concentration accelerates. As indicated in Table 2, the daily iron requirement has been placed at 1.8 mg. In the adolescent girls, the onset of menstruation at the end of the growth spurt increases iron losses. Hallberg *et al* (1966) estimated that in a 15 year-old girl, menstrual losses are less than in older women and the average daily losses (Baker and DeMaeyer, 1979) do not exceed 1.4 mg in 95% of individuals. An iron requirement of 2.4 mg per day has therefore been recommended (WHO, 1972).

As discussed earlier, many millions of people in the world suffer from parasitic infestations which could lead to severe iron losses. For these individuals, iron requirement would be higher than those recommended in Table 2.

4.1.4 Dietary Iron and its Absorption

The absorption of iron is a function of the quantity of iron present in the diet, the bioavailability of the dietary iron, and the iron status of the individual. Each of these factors will be briefly discussed.

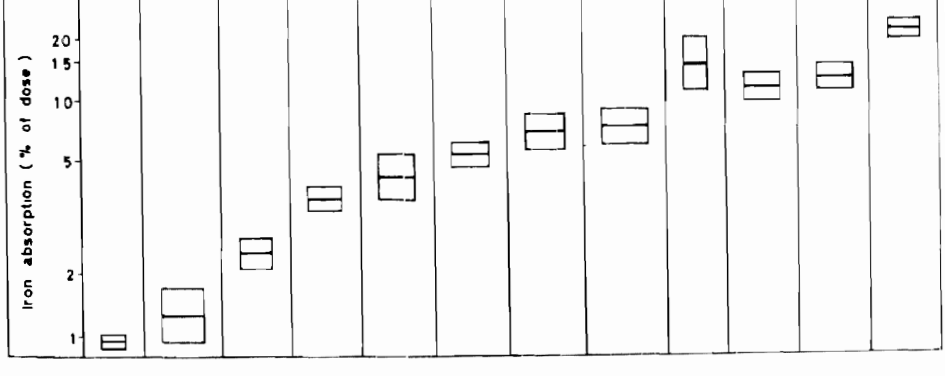
In the past, it has been customary to focus only on dietary iron content. Iron intake of various communities have been determined through dietary surveys (such surveys have been extremely rare in Malaysia) where iron ingestion has been estimated from calculations based on iron content of foods as reported in food composition tables or by direct chemical measurements of iron in the foods consumed. Quite different results may be obtained from the two methods of estimation, depending, especially, on the way foods are handled. It is thought that the higher results obtained by direct analysis of prepared ready-to-eat meals is due to the presence of extrinsic iron in the diet, coming mainly from food containers, other surfaces and contaminating dirt and earth (Bothwell *et al*, 1979). However, with proper food handling, estimates of iron content from tables and chemical analysis would be quite close and in fact, values obtained by the latter method may even be lower. An idea of the dietary iron content of local foods may be obtained from the food table complied by the IMR (Tee, 1982).

While the content of iron in the food is obviously important, it is now clear that of even greater nutritional significance is the bioavailability of dietary iron. Bioavailability in turn depends on various factors, the most important being the nature of the iron and the combination of foods in the diet.

Food iron exists primarily in the non-heme form of inorganic iron III (ferric) complexes which are broken down during digestion, the iron being partly reduced to the more readily absorbed iron II (ferrous) form. A lesser amount of food iron is present in the heme proteins, hemoglobin and myoglobin, which are present in foods of animal origin. Heme is split from the globin portions of these two pigments and assimilated intact by the intestinal mucosa, and ionic iron released by a heme splitting enzyme within the mucosal cell (Dallman, Siimes and Stekel, 1980). Studies carried out in the past 10 years or so on iron absorption from foods labelled with radioactive iron have contributed a great deal to our understanding of this complex biological process. Using these techniques, it has been amply demonstrated that heme iron in the diet is easily absorbed whereas non-heme iron is of a very much poorer bioavailability (Cook *et al,* 1972; Layrisse and Martinez-Torres, 1972; Hallberg and Bjorn-Rasmussen, 1972; Bjorn-Rasmussen *et al,* 1974; Aung-Than-Batu, Thien-Than andThane-Toe, 1976; Layrisse, 1980). Figure 4 (from WHO, 1972) clearly shows the differences in iron absorption from different foodstuffs.

Figure 4. Iron Absorption From Food*

	FOOD OF VEGETABLE ORIGIN							FOOD OF ANIMAL ORIGIN				
	Rice	Spinach	Black beans	Corn	Lettuce.	Wheat	Soya beans	Ferritin	Veal liver	Fish muscle	Haemo-globin	Veal muscle
Dose of food Fe	2 mg	2 mg	3-4 mg	2-4 mg	1-17 mg	2-4 mg	3-4 mg	3 mg	3 mg	1-2 mg	3-4 mg	3-4 mg
No cases	11	9	137	73	13	42	38	17	11	34	39	96



* Collaborative study of the Department of Botany and Medicine, University of Washington at Seattle, USA, and the Department of Pathophysiology, Instituto Venezolano de Investigacions Clentificas, Caracas, Venezuela. The horizontal thick line in the middle of the box represent the geometrical mean and the area in the box shows the limits of one standard error.

Source: WHO, 1972

As reviewed by Layrisse (1975), mean iron absorption from foods of plant origin eaten alone in both normal and iron deficient subjects could vary from 1% for rice and spinach, to 3% for maize and black beans, 4% for lettuce, 5% for wheat and going up to 7% for soya beans. On the other hand, in foods of animal origin, iron absorption ranges from 3% for eggs, 11% for fish, 12% for hemoglobin, to 22% for veal liver and muscle. It is however well known that foods of animal origin are frequently not present in the diets of poorer communities so that only a small proportion of the iron in the diet is heme iron.

Of even greater significance than the nature of the dietary iron is the extent to which dietary iron availability can be altered by the presence of other ingredients in the meal (Layrisse, Martinez-Torres and Roche, 1968; Layrisse and Martinez-Torres, 1972). The variation in bioavailability (from −80% to +500%) is much greater than the variation in the amount of non-heme iron in the diet (Bothwell *et al,* 1979). In summarising results of various studies on the interaction of foods on iron absorption, these authors noted that the bioavailability of iron from plant origin (non-heme iron) is enhanced by ascorbic acid, fish and meat (striated muscle, liver) but not by milk or cheese. On the other hand, various dietary ingredients such as tannates (in tea and coffee), calcium phosphate, bran, and egg-yolk are reported to be able to reduce non-heme iron absorption. Phytic acid (e.g. from legumes) is also known to interfere with iron absorption and utilization through the precipitation of insoluble salts (Aykroyd and Doughty, 1964). Cook *et al* (1981) have recently reviewed the various biochemical enhancers and inhibitors of iron absorption.

Various studies in different parts of the world have been carried out to determine iron absorption from composite meals. Results of some such studies have been summarised by Baker and DeMaeyer (1979) where absorption of iron from diets (consisting mainly of foods of plant origin) ranged from about 2% to 12%. Hallberg (1981) has also summarised his experiences in this area. Dallman, Siimes and Stekel (1980) have discussed the absorption of iron in various forms of milk, infant foods and transitional or weaning foods.

It is thus clear that dietary iron content and the bioavailability of iron in the diet will determine the quantity of iron available to the intestine for absorption. The intestinal mucosa, however, does not necessarily take up all the available iron. Absorption will be determined by the iron status of the body: absorption normally decreases as stores increase and increases as stores become depleted (Heinrich, 1970; Aisen, 1982). It has been estimated that the male, with his stores of about 1000 mg absorbs about 6% of the iron in the average American diet whilst the female, because of her lower reserves of about 300 mg, absorbs up to 15% of the dietary iron (Bothwell *et al,* 1979).

4.1.5 Dietary Iron Requirements

It is thus clear that in considering the dietary iron requirements of individuals, allowances must be made for the rate of absorption of iron from the diet. A Joint FAO/WHO Expert Group (WHO, 1970) met to review information about iron absorption from mixed diets and revised the previous recommended daily dietary iron intake (WHO, 1959). This later report recomended that estimates of daily dietary iron requirements be based on three types of diets with different proportions of foods of animal origin, and thus differing degrees of iron absorption as shown in Table 3.

Table 3
Absorption of Iron from Diets Containing Different Proportions of Foods of Animal Origin

Type of diet	Assumed upper limit of iron absorption by normal individuals
Less than 10% of calories from foods of animal origin	10%
10-25% of calories from foods of animal origin	15%
More than 25% of calories from foods of animal origin	20%

Source: WHO (1970)

Based on these considerations, recommended daily intakes of iron were retabulated according to these 3 categories of foods. A subsequent review by a WHO Expert Group (WHO, 1972) made slight modifications to these recommendations; these are given in Table 4.

Table 4. Recommended Daily Intakes of Iron

Group	absorbed iron required (mg)	mg of iron required, according to diet		
		Animal foods below 10% of calories[a]	Animal foods 10-25% of calories	Animal foods over 25% of calories[a]
Infants 0-4 months	0.5	-[b]	-	-
Infants 5-12 months	0.7	7	5	4
Children 1-12 years	1.0	10	7	5
Boys 13-16 years	1.8	18	12	9
Girls 13-16 years	2.4	24	16	12
Menstruating women	2.8	28	19	14
Men	0.9	9	6	5
Pregnancy, 1st half	0.8	8[c]	5	4
2nd half	3.0	30	20	15
Lactation	2.4	24	16	12

[a] In populations eating virtualy no foodstuffs of animal origin, the intakes need to be higher.
[b] Breast-feeding is assumed to be adequate.
[c] Values of dietary iron intake for pregnancy and lactation calculated by author by multiplying amount of absorbed iron required by a factor of 10 for the 1st type of diet, 6.7 for the 2nd type and 5 for the 3rd type of diet. Table retabulate from : WHO (1970 and 1972) and Baker and DeMaeyer (1979).

In making these recommendations, the WHO group (1972) had pointed out that:

(1) the recommended intakes would be adequate in all but 5% of cases;
(2) it is assumed that the requirements of calories and of all other nutrients are fully met;
(3) there are no specific recommended intakes for population groups eating virtually no foodstuffs of animal orgin, in whom intake would have to be raised considerably; and
(4) the recommended intakes do not take into account any additional requirement imposed by unusual environment stress, by disease or by parasitic infections.

4.2 FOLATE BALANCE

4.2.1 Folate Stores

Folate is said to be present in most body tissues and fluid and is particularly high in liver (Romine, 1960). There are no good estimates of the

total extent of body stores (Baker and DeMaeyer, 1979). Chanarin (1979) had however reported a total folate content of 0.7 - 17 µg/g in the normal liver. It has also been shown (Herbert, 1962b) that when intake of dietary folate was reduced to a very low level, body stores of folate were sufficient to meet the usual requirements of the body for several months. Substantial amounts of folate are synthesized by microorganisms in the large gut but this is not available for absorption (Masawe, 1981). In the fetus, the main stores of folate appear to have been laid down in the last trimester of pregnancy. Premature infants therefore may be expected to have smaller stores than full-term infants (Layrisse, Roche and Baker, 1976).

4.2.2 Folate Losses

Folate is removed from the blood by the liver and secreted in the bile at a higher concentration than is present in the serum (Baker, Kumar and Swaminathan, 1965) but the majority of this folate is probably reabsorbed (Baker and DeMaeyer, 1979). Normally, some folate is excreted in the stools (Layrisse, Roche and Baker, 1976), but this is probably derived from bacterial synthesis in the colon and does not represent true excretion from the body folate pool (Baker and DeMaeyer, 1979). Folate is also known to be excreted in urine, but the daily urinary excretion, as reviewed by Metz (1963) amounts to less than 10µg.

4.2.3 Folate Requirements

It is well established that folate is utilized by the body in the intermediary metabolism of purine and pyrimidine nucleotides, an essential step in the synthesis of nucleic acids (see for example review of folic acid metabolism by Stockstad and Koch, 1967; Chanarin, 1979). Hence, body folate requirements vary with these metabolic processes and form the major factor determining the daily folate requirements of normal individuals (Baker and DeMaeyer, 1979; Masawe, 1981).

Estimates of the daily requirements for folate in the adult have basically been made in two ways; (a) studying the hematological response of folate deficient subjects to pteroylglutamic acid or other folate compounds; (b) studying indices of folate nutrition in subjects on controlled folate intakes (Baker and DeMaeyer, 1979). Somewhat different results have been reported by various research groups on the amount of folate required to bring about hematological responses to folate deficient subjects; values ranged from 50-200 µg (Zalusky and Herbert, 1961; Hansen and Weinfeld, 1962; Herbert, 1962a). Hence, WHO (1970) had recommended a daily dietary intake of 200 µg of "free" (i.e. measurable by *Lactobacillus casei* assay) folate for adults. However, it was recognized that the polyglutamate forms of folate in the food is also available for absorption although the relative availability of the free and polyglutamate forms appears to be unclear (WHO, 1972; Baker

and DeMaeyer, 1979) (see Section 4.2.4 on Dietary Folate and its Absorption). Some adjustments were hence made and the WHO Expert Group on Nutritional Anemias (WHO, 1972) recommended a daily intake of 400 µg of total folate for the adult (given in Table 5 as 13 years and above).

Table 5. Recommended Daily Intakes of Folate

group	µg of total folate*
Age group:	
0 - 6 months	40 - 50
7 - 12 months	120
1 - 12 years	200
13 years and over	400
Pregnant women	800
Lactating women	600

*"Total folate" includes "free" and "bound" or polyglutamate forms

Source: WHO, 1972

Evidences appear to point to the need for increased folate requirements during pregnancy. Studies by various research groups have shown that:

(a) pregnant women unsupplemented with folate showed a progressive fall in serum and red cell folate concentrations (Ball and Giles, 1964; Hansen, 1967; Whiteside, Unger and Cowling, 1968; Temperley, Meehan and Gatenby, 1968; Davis, Stenhouse and Woodliff, 1969);

(b) as pregnancy progresses, intravenously administered pteroylmonoglutamic acid is cleared more rapidly from the blood; this is particularly so in women with twin pregnancy (Chanarin *et al*, 1959);

(c) megaloblastic changes in the bone marrow of pregnant women are common in many communities, particularly the economically deprived (see Section 7 on Prevalence).

Methodologies for the estimation of folate requirement during pregnancy vary somewhat from those used for adults and non-pregnant subjects. The best estimates are said to be those based on determinations of the amounts of folic acid required to prevent the development of evidences of deficiency, such as a fall in serum and red cell folate concentrations (Baker and DeMaeyer, 1979). Differing amounts that can achieve this have been reported: 100 µg by Hansen and Rybo (1967), (b) 300 µg by Willoughby and coworkers (Willoughby and Jewell, 1966; Willoughby, 1967; Willoughby and Jewell, 1968). Considering these evidences, WHO (1970) has recommended a daily dietary intake of 400 µg of "free" folate throughout pregnancy. A subsequent WHO report (WHO, 1972) revised this and gave a recommended daily intake of 800 µg of total folate during this period.

During lactation, extra folate (over that in the nonpregnant individual) is needed to meet amounts excreted in the breast milk (about 50 µg/litre) plus possibly other metabolic requirements of the lactating mother (WHO, 1970). It was thus recommended that for a nursing mother, a daily intake of 300 ug "free" folate was needed (WHO, 1970), which was later revised to 600 µg total folate (WHO, 1972).

In view of the metabolic functions of folic acid, it would be expected that the daily requirement of this vitamin would be relatively increased during periods of rapid growth of the infant and young child. There is however relatively little information on the folate requirements of these two groups (Baker and DeMaeyer, 1979). For the first 6 months of life, breast milk (containing about 50 µg folate per litre) appears to provide adequate amounts of folate; WHO (1972) has therefore recommended a daily intake of 40-50 µg for this early period of infancy. From 7 to 12 months, 120 µg total folate per day was recommended and from 1 to 12 years, 200 µg of total folate. For adolescents of 13-19 years, the same intake as for adults, i.e. 400 µg per day was recommended (see Table 5).

4.2.4 Dietary Folate and Its Absorption

Data on the occurrence of folate in foods are relatively scarce, compared with the other vitamins. Locally, there has been no report of folate determination of Malaysian foods. Elsewhere, one of the most valuable reference appears to be that of Toepfer *et al* (1951), which listed the folate content of some 348 foods. However, since the publication had appeared over 25 years ago, recent understanding of the properties of this vitamin may give rise to rather different results. Other more recent tables of folate content of foods are those of McCance and Widdowson (1961) and Hoppner, Lampi and Perrin (1972). It appears that folate occurs in a wide variety of plant and animal foodstuffs. Meat (especially liver), leafy vegetables (such as spinach, asparagus) and yeast are said to be particularly rich sources (Chanarin, 1979; Malin, 1975).

Studies on the effect of food preparation on its folate content (as reviewed by Malin, 1975 and Chanarin, 1979) have shown that many of the occurring folates are extremely labile and easily destroyed by prolonged cooking. Thus, estimates of dietary intake using calculations from Food Tables would give erroneous results. Clearly, investigations of dietary intakes of communities must include measurements of folate in prepared foods as actually eaten in the homes. The review of Chanarin (1979) of the various studies of such diets shows a considerable amount of variation. Baker and DeMaeyer

(1979) felt that some of these variations were due to methodological differences and suggested the adoption of standardised techniques so that meaningful comparison may be made between dietaries in different parts of the world. According to Hoffbrand (1878), the normal adult dietary intake of folate ranges from about 400 to 1000 µg, of which 50-80% is absorbed.

The methods for quantitating and differentiating the different forms of folate in food have been based mainly on differential microbiological assays, combined with chromatography (see for example Herbert and Bertino, 1967). Three assay organisms have commonly been used, namely, *Lactobacillus casei*, *Streptococcus faecalis* and *Pediococcus cerevisiae*. It is generally accepted that the *L. casei* assay which measures all the known pteroylglutamates containing up to 3 L-glutamic acid residues (including the important 5-methyl tetrahydrofolate) is the most useful (Herbert and Bertino, 1967).

Folate in food has been classified into two main groups according to its availability to *L. casei* (WHO, 1970; Baker and DeMaeyer, 1979):

(a) the so-called "free" folate: these are directly available for *L. casei* assay and may contain up to 3 glutamic acid moieties, i.e. the glutamates, diglutamates and triglutamates;

(b) "bound" folates or those polyglutamates with more than 3 glutamic acid moieties that are available to *L. casei* only after treatment with the enzyme conjugase (gammaglutamyl peptidase).

Hence the term "total" folate would include both the "free" folate and "bound" folate. Few recent data exist on the amount of "free" and "total" folate in different foodstuffs. The recent Table of Hoppner, Lampi and Perrin (1972) however contains information on the "free" and "total" folate content of many Canadian foods. The few reports available indicate that the folate in vegetables other than lentils is generally in the conjugated form. On the other hand, the large quantity of folate in mammalian liver (7-20 µg per g) is almost entirely in the "free" form (WHO, 1970).

Some idea of the folate content of Western type of prepared meals has been provided by Butterworth, Santini and Formmeyer (1963), Hurdle (1968a and b), and Chanarin *et al* (1968). A mean intake of "free" folate of about 160 µg and of "total" folate of 680 µg per day has been reported by Chanarin *et al* (1968). One would however expect that this ratio would depend on the proportion of vegetable and animal foods in the diets, and hence such estimates may not apply for our diets.

Absorption of pterylmonoglutamic acid is thought to occur throughout

the human small intestine, but is maximal in the upper jejunum and occurs against a concentration gradient, suggesting an active transport mechanism (Hepner *et al,* 1968). Using C^{14} labelled polyglutamates, it has been demonstrated that these are broken down during absorption chiefly to monglutamates (Butterworth, Baugh and Krumdieck, 1969; Baugh *et al,* 1971), presumably by the action of intestinal conjugase (Baker and DeMaeyer, 1979). More detailed reviews of the absorption and malabsorption of folic acid have been made by Berstein *et al* (1970) and Rosenberg (1976).

There appears to be some controversy regarding the availability of polyglutamate forms of folate as compared to pteroylmonoglutamic acid. Monitoring urinary excretion of folate (Swendseid *et al,* 1947; Rosenberg and Goodwin, 1971; Tamura and Stokstad, 1973) or plasma *L.casei* activity (Hoffbrand *et al,* 1969) following feeding mono and heptaglutamates, it has been observed that similar results have been obtained with either forms. Using a slightly different approach, Grossowicz, Rachmilewitz and Izak (1972, 1975) found that the rise in serum folate concentration, after administering yeast extracts containing polyglutamate folate was similar to or greater than the rise of serum folate concentration obtained by feeding equimolar amounts of pteroylmonoglutamic acid. On the other hand, Jeejeebhoy *et al* (1968) and more recently, Perry and Chanarin (1972) reported a higher rise in serum folate when pteroylmonoglutamate folate was fed to subjects than when a similar amount of yeast polyglutamate folate was given. An earlier report by Perry and Chanarin (1968) showed that when either mono or heptaglutamates was fed as a folate supplement to volunteers over a 3-month period, the rise in red cell folate concentration was 45% higher in the group fed the monoglutamate. It was suggested that heptaglumate was utilized to one-third of the extent of monoglutamate. Reviewing the available evidences, Baker and DeMaeyer (1979) felt that the reasons for the discrepancies in the reported results could be due to:

(a) the use of yeast, which is not a usual food item, as a source of heptaglutamate folate by some research groups;
(b) the presence of some conjugase inhibitor in the polyglutamate given to the subjects;
(c) the widely differing doses of folate used in the experiments, since the relative absorbability may differ at different dose levels.

Baker and DeMaeyer (1979) emphasized the need for further work to be done in this area. It was further suggested that in the meantime, from the nutritional viewpoint, it would probably be safer to regard the polyglutamate froms as possibly less available than an equimolar amount of pteroylmonoglutamic acid.

4.3 VITAMIN B_{12} BALANCE

4.3.1 Vitamin B_{12} Stores

Like folate, vitamin B_{12} is present in all body tissues, but the main concentrations are in the liver and the kidney (Baker and DeMaeyer, 1979). Body stores are believed to be laid down in the fetus during development and at birth, the liver contains a total of some 20-25 µg of the vitamin (Baker *et al,* 1962). The stores enlarge as the individual grows, such that in healthy adults on a diet relatively rich in vitamin B_{12} an average content of 0.91 µg/g of liver tissue has been reported (Joske, 1963). Assuming an average liver weight of 1700g, this amounts to a total of 1.5 mg (Baker and DeMaeyer, 1979). Studies cited by WHO (1970) indicate that total body stores of vitamin B_{12} may range from 0.8 to 12 mg.

4.3.2 Vitamin B_{12} Losses

As with folate, vitamin B_{12} is likewise excreted in the bile. The majority of this vitamin combines with intrinsic factor and is reabsorbed from the intestine (Halsted *et al,* 1956; Grasbeck, Nyberg and Reizenstein, 1958). The amounts of the vitamin excreted in the urine are said to be small - about 0.1 to 0.2 µg per day (Mollin and Ross, 1952). Stools are known to contain relatively large amounts of vitamin B_{12} but the majority of this is derived from *de novo* synthesis by colonic bacteria and is unavailable to the individual (Baker and DeMaeyer, 1979). Megaloblastic anemia has been observed to occur on an average of 4 years after total gastrectomy, suggesting a loss or utilization of over 1 µg of vitamin B_{12} per day. At the same time, studies of the whole body turnover of the vitamin using B_{12} labelled with a radioactive isotope of cobalt have shown that the overall loss of the vitamin from the body is exponential at the rate of about 0.1 to 0.2% of the total body content per day, such loss occurring irrespective of the size of the stores (Grasbeck, 1960; Heyssel *et al,* 1966; Adams and Boddy, 1968).

4.3.3 Vitamin B_{12} Requirements

Daily intake of vitamin B_{12} for adults is 2 µg (Table 6) as recommended by WHO (1970); this figure had remained unchanged in a subsequent report (WHO, 1972). This figure was calculated based on data from various clinical studies, body stores of vitamin B_{12} and turnover studies. It was thought that if the percentage loss of vitamin B_{12} from the body is unaffected by the level of its stores, then one could calculate the minimum daily requirement of the vitamin from the turnover of radioisotope labelled vitamin B_{12} in patients on the point of hematological or clinical relapse. Data from the study of Anderson (1965) was particularly considered for this calculation by the WHO Expert Group (WHO, 1970). The Group considered data from a group of patients suffering from malabsorption of the vitamin in whom the

hematological status was normal or above the range usualy found in patients with even minimal signs of deficiency, i.e. serum vitamin $B_{12} > 130$ pg per ml. However, since this level was below 200 pg per ml, the level taken to exclude deficiency in the individual patient, they were considered to be approaching relapse. These patients were estimated to have an average total body B_{12} content of 525μg. Assuming the daily loss of the vitamin to be between 0.1 and 0.2% per day, the amount of vitamin which must be absorbed to maintain stores at this level would be 0.5 to 1.0μg per day. However, this would not be sufficient to maintain serum vitamin B_{12} concentrations at or above the suggested level of 200 pg per ml, and because of the known biological variations between individuals and the possibility that the ordinary stresses of daily life may increase the physiological needs for the vitamin, the Group recommended a daily intake of 2μg for the normal adult.

Table 6. Recommended Daily Intakes of Vitamin B_{12}

Group	μg of vitamin B
Age group:	
0 - 12 months	0.3
1 - 3 years	0.9
4 - 9 years	1.5
10 years and over	2.0
Pregnant women	3.0
Lactating women	2.5

Source: WHO, 1970, 1972

There is undoubtedly an increased requirement for vitamin B_{12} in pregnancy, at least partly due to the fetal drain on maternal stores (WHO, 1970). Since the liver contains about 25μg of the vitamin (Baker *et al,* 1962), the total body content of the fetus would probably be about 30μg (Baker and DeMaeyer, 1979). This would entail the transfer from mother to the fetus of about 0.2μg/day during the latter half of the pregnancy. Considering this and the expected increased metabolic demand in pregnancy, the WHO Expert Group concluded that an additional daily intake of 1.0μg of vitamin B_{12} is needed, thus making the recommended daily intake of vitamin B_{12} in pregnancy to 3.0μg (WHO, 1970) (see Table 6).

During lactation, vitamin B_{12} is secreted into breast milk at a concentration similar to or a little lower than the mean level of 450 pg per ml in plasma (Baker *et al,* 1962). Hence, approximately 0.3μg of the vitamin per day may be lost in the milk. An extra intake of 0.5μg per day over the daily intake for adults was thus recommended by the Expert Group (WHO, 1970), making the amount to 2.5μg per day.

In mothers with normal body vitamin B_{12} stores, breast milk supplies 0.3 μg of the vitamin daily, which is clearly adequate, and the meagre

evidence available suggests that the milk from mothers with slightly lower stores would also be adequate (WHO, 1970). It was thus recommended that the intake for infants be 0.3 μg of B_{12} daily. Considering that vitamin B_{12} deficiency does not occur in normal children on adequate calorie and animal protein intakes, the WHO Expert Group thus based the calculation of the daily desirable intake of the vitamin on the recommended calorie intakes for the different age groups (see Table 6).

4.3.4 Dietary Vitamin B_{12} and its Absorption

The best dietary sources of vitamin B_{12} are liver, meat and other animal products (Chung *et al*, 1961). Rothenberg and Cotter (1978) have given a simple classification of foods with high, medium, moderate and low amounts of the vitamin. According to Chung *et al* (1961), the amount of the vitamin in a selection of American diets varied with increasing cost from 1 to 85 μg daily. Data on dietary intake of vitamin B_{12} in developing countries is extremely scarce.

The mechanism for vitamin B_{12} absorption appears to be a complex process requiring the interaction of a number of specific macromolecules (Stenman, 1976; Rothenberg and Cotter, 1978). It involves a preliminary step of releasing the usually protein-bound vitamin by cooking or by the action of digestive enzymes in the stomach. The freed vitamin then combines the first specific macromolecule, the intrinsic factor secreted by the gastric parietal cells. The complex of vitamin B_{12} and intrinsic factor is adsorbed to the brush border of the ileum by a second specific macromolecule. The vitamin is then absorbed across the ileum mucosal cells. This appears to be the manner most of the ingested vitamin B_{12} is absorbed although a very small proportion of the vitamin may also be absorbed by a passive diffusion process (Corcino, Waxman and Herbert, 1970).

It is further known that the largest amount of vitamin B_{12} that can be absorbed from any one meal by the dominant mechanism is limited to about 1.5 to 3.5 μg (WHO, 1970). It would appear therefore that the usual non-vegetarian Western diet provides more than enough vitamin B_{12} to meet the estimated daily requirements (Baker and DeMaeyer, 1979). It has also be recently shown that some bacteria isolated from the lumen of the small intestine of subjects can produce significant amounts of vitamin B_{12} (Alpert, Mathan and Baker, 1980). As discussed under 4.3.2, B_{12} is known to be produced by bacteria in the colon; but this is unavailable to the individual. Since it is also known that free intrinsic factor in biological active form may reach the small intestine (Kapadia, Mathan and Baker, 1976), endogenously produced vitamin B_{12} may be absorbed and contribute to the individual's daily vitamin B_{12} requirements (Baker, 1981).

5 ETIOLOGY AND PATHOGENESIS

5.1 ETIOLOGY OF IRON DEFICIENCY

As discussed earlier, iron balance in a healthy, adequately nourished individual may be upset by a number of factors. An imbalance can occur as a result of a reduction of iron absorption, an increase of iron requirement or an increase of iron losses. Consequently, iron supply to the bone marrow for normal erythropoiesis is impaired. This is the basic etiology of iron deficiency.

A reduced absorption of iron into the body can occur as the result either of low levels of dietary iron or a poor biological availability of dietary iron, or both. These are important causes of iron deficiency anemia in developing countries where the economy is restricted, so that the diets are commonly made up almost wholly of rice or maize staple and very little foods of animal origin. Similarly, dietary inadequacy amongst vegetarians would be an important factor. At certain periods of life, such as during the rapid growth of the young child or pregnancy, there is an increase in the requirement for iron. Having too many babies too frequently would further aggravate the situation in the case of the pregnant women. Increased amounts must thus be absorbed to maintain the iron balance, or deficiency state would result. Again, this is an important cause of the widespread anemia seen in young children and pregnant women. Increased iron losses can occur during menstruation as well as with parasitic, particularly hookworm, infections. Similarly, these losses, if not made up by increased absorption of iron, would result in an imbalance in the body. These have been discussed in Section 4.1.2 on "iron losses".

It can be seen from the above outline that in the physiological sense, three groups of people are particularly vulnerable to iron deficiency, namely the young child, the menstruating woman, and the pregnant woman. These are the times when iron balance is particularly precarious. Because of increased requirements and/or losses, these individuals would have to increase their iron intake to maintain the balance in the body. However, due to low iron content in their diet and/or poor bioavailability of the dietary iron, or as Harrison (1982) puts it, a diet (which is what most people in the tropics can afford due to mass poverty) with "low-meat, low-vitamin, high-carbohydrate, high-phytate content", these requirements are very often not met. It is therefore not surprising that there is a high prevalance of iron-deficiency anemia amongst these vulnerable groups in many communities all over the world, especially the developing nations. Harrison (1982) further points out that it is due to the depth of socio-economic deprivation in the tropics (emphasizing on the high illiteracy rates) that severe anemia is so common and its effects so serious.

This is of course a very simplified view of the etiology of iron deficiency anemia. It is, however, most important from the public health point of view. Nevertheless, it would be essential to bear in mind that there are numerous other factors that can bring about a deficiency state, such as malabsorption due to intestinal disorders, excessive blood loss due to various anatomical lesions, intramuscular hematolysis and hemoglobinuria (see for example Fairbanks, Fahey and Beutler, 1971; Fairbanks, 1978).

The imbalance of iron nutrition in the body could of course also mean a positive balance or iron overload. Several recent publications have reviewed this (e.g. Bothwell *et al,* 1979; Halliday and Powell, 1982, Bothwell and Charlton, 1982; Finch and Huebers, 1982). It is a relatively rare disorder in most countries and would not be discussed in this review.

5.2 PATHOGENESIS OF IRON DEFICIENCY

Iron deficiency arises when, over a sufficient period of time, the need for iron exceeds its availability. It evolves slowly and progresses through several stages before it develops into frank anemia. It has commonly been divided into 3 stages Council on Foods and Nutrition, 1968, Cook, 1982);

(1) The earliest stage is the storage iron depletion or prelatent stage, where iron reserves are reduced but there is not yet a decrease in iron supply to the developing red cells. Hence, serum ferritin levels may be lowered, but serum iron and transferin saturation, free erythrocyte porphyrin as well as hemoglobin remain normal.
(2) The second and more advanced stage, referred to as the iron deficient erythropoiesis or latent stage, is characterised by a diminished supply of iron to the erythroids, but the circulating hemoglobin is not yet significantly decreased. Thus, iron stores would be almost depleted (low serum ferritin value), there would be a reduction in transferrin saturation level, as well as an increase in free erythrocyte porphyrin. Hemoglobin concentrations however remain normal.
(3) The third or final stage is the overt iron deficiency anemia or manifest iron deficiency stage. All the above-mentioned hematological indices, including hemoglobin, would be abnormal.

The above outlined sequence of events and changes has been clearly depicted diagrammatically by Cook and Finch (1979).

5.3 ETIOLOGY AND PATHOGENESIS OF MEGALOBLASTIC ANEMIA

Megaloblastic (large germ cell) anemia is a morphological entity of abnormal hemopoiesis characterised in the red cell line by increased cell size with finely stippled appearance of nuclear chromatin and asynchronous

nuclear and cytoplasmic development. Megaloblastosis is the morphological expression of deranged DNA synthesis resulting from, in the majority of patients, deficiency of vitamin B_{12} folate or both (Das and Herbert, 1976). The principal causes of megaloblastic anemia have been conveniently summarised and tabulated by Chanarin (1976). The etiology and pathogenesis of deficiencies due to these two vitamins are separately discussed below. It should however be borne in mind, as pointed out by Das and Herbert (976), that there are closely interrelated metabolic roles of these two vitamins in the pathogenesis of megaloblastic anemia.

5.3.1 Etiology and Pathogenesis of Folate Deficiency

A combination of factors leads to negative folate balance or folate deficiency. A convenient tabulation of the various causes of folate deficiency is given in Hoffbrand (1978). Poor diet is usually the major cause, since few diseases cause malabsorption or excess of folate utilization of such severity that a good intake of the vitamin cannot overcome losses (Hoffbrand, 1978). Hence communities which have a diet with no rich sources of folate, and especially those who cook their food for prolonged periods, as is the custom in South India (Baker, 1981) would be particularly prone to develop folate deficiency.

During pregnancy, fetal and uterine growth together with the expansion of the maternal blood volume result in an increased demand for iron (as discussed earlier) as well as for folic acid (Harrison, 1982). Since body stores of folate are limited, the prevalence of folate deficiency in pregnancy would depend mainly on the dietary intake of women during pregnancy (Baker, 1981). As noted by Yusufji, Mathan and Baker (1973), 66% of the unsupplemented South Indian pregnant women of lower socio-economic strata had a megaloblastic bone marrow at term. Hence dietaries lacking in folate certainly cannot meet this increased demand and deficiency frequently develops.

Increased requirements for folate also occur during periods of rapid growth of the infants and young children. These extra needs are not likely to be met with if the diet of the family is poor. Deficient intake of folate is said to be most often associated with artificial feeding of infants, and processed weaning foods, unless supplemented, are particularly liable to be low in folate (Layrisse, Roche and Baker, 1976).

Severe folate deficiency many also occur in tropical sprue, gluten-induced enteropathy, and a congenital specific folate malabsorption, despite a normal dietary folate content (Hoffbrand, 1978). Tropical sprue has been defined as "an idiopathic malabsorption syndrome occurring among residents of or visitors to certain areas of the tropics" (Klipstein and Baker, 1970). Although it has not been widely studied in some countries, it is pro-

bably present throughout most of tropical Asia (Baker, 1981). Its etiology is at present unknown, but it occurs both in endemic and epidemic forms and it is thought that at least one of the causes may be an infectious agent (Baker, 1972). Absorption of pteroylmonoglutamic acid in patients with tropical sprue appears to be normal, but malabsorption of polyglutamate is common. The precise biochemical defect responsible for this however remains to be elucidated (Baker, 1981).

5.3.2 Etiology and Pathogenesis of Vitamin B_{12} Deficiency

Theoretically, vitamin B_{12} deficiency would result from dietary deficiencies in communities where the intake of animal protein is low, for economic or religious reasons. Such diets have been described by Rothenberg and Cotter (1978) for some parts of India. It has been further noted by Baker (1981) that of all the Asians, the Indians of the lower socio-economic strata have the lowest dietary intake of vitamin B_{12}: the South Indian vegetarian diets provide only 0.3 to 0.5 μg of the vitamin per adult per day (Baker and Mathan, 1918). However, although serum levels of the vitamin are lower than in the West (Yusufji, Mathan and Baker, 1973), and liver stores are also low, overt vitamin B_{12} deficiency of purely dietary origin is said to be surprisingly uncommon (WHO, 1970; Rothenberg and Cotter, 1978; Baker, 1981). It is thought that the bulk of daily intake of the vitamin for these Indians could have been derived from bacterial contamination of food and water (Baker, 1981). On the other hand, the prevalence of nutritional vitamin B_{12} deficiency anemia amongst vegetarian Indian immigrants to the United Kingdom appears to be high (Britt, Harper and Spray, 1971). Baker (1981) postulated that this is a result of migration to a more sanitized environment, wherein the lower degree of bacterial contamination of the water and food reduces the vitamin B_{12} intake and also leads to a reduction in the bacterial population of the small intestine, resulting in a decreased endogenous production of the vitamin.

Vitamin B_{12} deficiency, usually in association with folate deficiency is a frequent complication of pregnancy in India and Southeast Asia (WHO, 1970). This deficiency is probably due to poor diet, increased demands and malabsorption (see below).

Infants born of vitamin B_{12} deficient mothers start their extra-uterine life with lowered body stores (Baker *et al*, 1962). If the daily intake of the vitamin from their mother's breast milk is insufficient for their metabolic needs, their body stores will be progressively depleted (Baker, 1981). Six of such infants (between 7-12 months old) were described by Jadhav *et al* (1962) and all had some of the characteristic clinical symptom of vitamin B_{12} deficiency. According to Baker (1981), anemia in such infants born of and suckled by vitamin B_{12} deficient mothers is perhaps the commonest form of dietary vitamin B_{12} deficiency anemia in infants.

Intestinal malabsorption as a result of frequently unsuspected tropical sprue could be an important cause of vitamin B_{12} deficiency in India and some countries in Southeast Asia; the respective contributions of dietary deficiency and malabsorption are however uncertain (WHO, 1970). The vitamin B_{12} deficiency in tropical sprue is mainly due to interference with absorption, and in the majority of patients, malabsorption of the vitamin is not corrected by the addition of intrinsic factor, suggesting the presence of an intestinal defect (Baker, 1981). The precise mechanism however is uncertain.

In "idiopathic" anemia described by Addison in 1855 and later called pernicious anemia (Castle, 1970), vitamin B_{12} deficiency occurs as a result of a lack of secretion of intrinsic factor, associated with an idiopathic (that is, not secondary to any other known disease) atrophy of gastric mucosa (Baker, 1981). According to Baker (1981), classical pernicious anemia probably does occur in Asians but with a very low prevalence.

The fish tapeworm *(Diphyllobothium latum)* is known to absorb vitamin B_{12} contained in food and can interfere with its absorption, thereby producing vitamin B_{12} deficiency (WHO, 1970). How far the presence of other helminths interfere with the absorption of vitamin B_{12} is uncertain.

5.4 ETIOLOGY OF ANEMIA IN MALAYSIA

In this country, the anemia problem was most frequently studied amongst pregnant women. Early investigators had already touched on the causes of anemia amongst these women. Corke and Bush (1930) felt that the condition had originated from "a combination of a blood-destroying infection such as malaria or syphilis and an insufficiency of vitamins in the diet". Tasker, Richadrdson and Llewellyn-Jones (1956) had also described briefly the poor dietary pattern of the most affected group, the Indians. It was clear that anemia in pregnant women was not directly caused by pregnancy, but merely aggravated by it (Corke and Bush, 1930 and Lourdenadin,1969). Ignorance, poor socio-economic conditions, large families and peculiar food habits of racial groups were the various factors that were believed to have contributed to the prevalence of malnutrition, which in turn resulted in the high incidence of nutritional anemia during pregancy (Lourdenadin, 1964 and 1969). Dietary patterns of these women were said to be poor, with a high carbohydrate, and low and erratic protein intake.

The etiologic factors giving rise to the two major forms of nutritional anemia were also well documented. Iron deficiency was believed to be result of a multiplicity of causes, including deficient intake, deficient absortion, increased metabolic demand during gestation, especially in repeated childbearing, and blood loss due to parasitic infection and menorrhagia (Llewellyn-Jones, 1965 and Lourdenadin 1964, 1965). In megaloblastic

anemia, folic acid was thought to play a greater role than vitamin B_{12}. Whilst poor dietary source of folic acid was thought to be a factor, poor absorption from the gut was not. To some extent, liver damage in malnutrition which hindered the conversion of folic acid to folinic acid, was thought to play a role (Llewellyn-Jones, 1965). Its high incidence amongst Indians was thought to be due to dietary inadequacy, which could have resulted from a low consumption of animal proteins and prolonged cooking of vegetables. There was however no simple cause and effect relationship between intake of folic acid and megaloblastic anemia (Lourdenadin, 1964, 1969).

5.5 HELMINTH INFESTATION IN THE ETIOLOGY OF ANEMIA IN MALAYSIA

The role of helminth infestation in the etiology of anemia has also been well studied. These will be reviewed in some detail. Early investigators in the country had already documented the association between helminth infestation and anemia. The reports of Battray (1918), Corke and Bush (1930) and Pallister (1934) in the early part of the century had touched on the incidence of helminthiasis in anemic subjects. In fact, most of the studies of the prevalence of nutritional anemia have also included examination of stool for these parasites. Some of the more recent reports that presented helminthic infestation prevalence are Chappel and Janowitz (1965), Kinzie, Kinzie and Tyas (1966), Lie-Injo and Virik (1966), Davis (1970) and Bisseru (1971). This association continued to be highlighted in the late 70s and more recent studies. Some of these are cited below to give an idea of the prevalence of such infestations in the communities.

The surveys of A.J.U. Anderson provide valuable information on the prevalence of intestinal parasites amongst children of various communities in Sarawak. The results of some of the surveys are summarised in Table 7 (results of the prevalence of anemia amongst these same children are given in Section 7 on Prevalence). In all communities, except the Penans in Gunong Mulu, ascaris was the most common helminth encountered, followed by trichuris and then hookworm. No hookworms were detected for the Malay group, said to be due to the fact that the study areas were constantly flooded by sea-water at high tides. Generally, it was the 1-8 years group who had the highest rate of infestation . Overall, infestation rates were high, and over 50% of the children were infested with one or more of these common intestinal parasites. Prevalence of helminthic infestation was particularly high for the Malays. The rates were said to have persisted despite treatment from time to time.

Table 7
Prevalence of Helminth Infestation Amongst Sarawak Children

Communities studied	% of children positive for			% of children positive for one or more of the parasites
	Ascaris	Trichuris	Hookworm	
Land Dayaks, Tebakang (Anderson, 1976a)				
<1 year (n = 8)	12	-	-	-
1 year (n = 12)	76	-	-	-
2-8 years (n = 181)	78	40	15.5	-
Iban, Middle Mukah River (Anderson, 1976b)				
<1 year (n = 16)	12.5	6.3	-	12.5
1 year (n = 12)	14.6	25.0	-	50.0
2-8 years (n = 102)	60.7	43.1	18.6	74.5
combined (n = 130)	53.0	36.9	14.6	64.6
Iban, Lemanak River (Anderson, 1977a)				
1-8 years (n = 114)	78.9	50.0	18.4	93.9
Iban, Sut & Mujong Rivers (Anderson, 1978a)				
<1 year (n = 13)	38.5	7.7	-	46.2
1 year (n = 32)	46.9	3.1	3.1	46.9
0-8 years (n = 248)	50.0	13.7	5.6	59.7
Malays, Sarawak River Delta (Anderson, 1977b)				
<1 year (n = 3)	100.0	66.6	0	100.0
1 year (n = 8)	75.0	25.0	0	75.0
2-8 years (n = 128)	93.8	80.5	0	96.9
Penan, Gunong Mulu (Anderson, 1978b)				
0-8 years (n = 81)	38.3	6.2	44.4	67.9
9+ years (n = 142)	28.2	7.7	50.7	69.0

Results of studies of the prevalence of anemia carried out by Kandiah and co-workers (Kandiah & Lim, 1976; Kandiah & Tan, 1967; Kandiah & Lim, 1977) will be discussed in Section 7.2. These studies also included stool examination for helminths. The results are summarised in Table 8. Comparisons of infestation rates between groups and communities are of course not strictly valid (especially comparison of rates reported by different investigators) because certain areas might have had recent treatment with antihelminths and there might be differences in techniques used by investigators. Among the intestinal helminths, only the soil-transmitted ones were found by Kandiah and coworkes. Of these, *Ascaris lumbricoides,*

Trichuris trichiura and *Necator americanus* were the most common. *Strongyloides* infestation was rare, and no *Enterobics* ova were found in the stools collected.

Table 8. Prevalence of Intestinal Parasites in two Malay Communities and an Indian Estate Population

Communities studied		% of subjects positive for			% subjects with multiple infestations		
		Ascaris	Trichuris	Hookworm	single	dual	triple
Malays, Ulu Jempol (Kandiah & Lim, 1976)							
pre-sch. child.	(n = 217)	23.9	9.1	10.0	—	5.8	0.5
school children	(n = 848)	20.6	22.6	6.0	—	5.8	0.5
others	(n=1293)	27.1	8.3	10.1	—	4.6	0.6
Malays, SLDA*Scheme, Ulu Rening (Kandiah & Tan, 1977)							
pre-sch. child.	(n = 51)	41.1	39.2	9.8	—	—	—
school children	(n = 58)	36.2	37.9	36.2	—	—	—
adult females	(n = 33)	33.3	36.4	60.6	—	—	—
adult males	(n = 17)	35.3	23.5	41.2	—	—	—
combined	(n = 159)	37.1	36.5	33.3	28	30	6
Indians, rubber estate, Selangor (Kandiah & Lim, 1977)							
pre-sch. child.	(n = 53)	22.6	28.3	11.3	—	—	—
school children	(n = 168)	43.5	47.0	13.7	—	—	—
adult males	(n = 73)	2.7	6.8	9.6	—	—	—
adult females	(n = 80)	11.3	23.8	21.2	—	—	—
combined	(n = 374)	25.7	31.0	14.2	—	—	—

SLDA*= State Land Development Authority

Similarly, the findings of Chong and co-workers (Chong *et al,* 1984) on various rural Malay communities in Peninsular Malaysia (Kota Baru, Kelantan; Mersing, Johore; Baling, Kedah and Perak Tengah, Perak) are summarised and tabulated in Table 9.

Table 9. Prevalence of Intestinal Helminths in Rural Malays

age group	n	% free of infestation	% with single infestation* ALO	TTO	HWO	% with triple infestation ALO TTO	ALO HWO	TTO HWO	% with double infestation ALO	TTO	HWO
Infants	43	81	9	0	0	5	0	0	5		
Pre-school children (both sexes)	298	29	22	15	4	19	4	1	6		
Primary school children (both sexes)	403	10	14	25	3	33	3	2	10		
Adolescent (both sexes)	133	13	18	18	1	28	5	5	12		
Adults (18-45 years)											
Males	112	31	16	16	11	13	2	4	7		
Females	287	22	17	13	9	17	7	4	9		
Senior Adults (>46 years, both sexes)	178	34	10	25	3	14	1	6	7		
Combined population	1,454	23	16	19	5	22	4	3	8		

*ALO - *Ascaris Lumbricodes ova*, TTO - *Trichuris trichiura* ova, HWO - Hookworm ova
Source: Chong *et al.* 1984

A recent study of children in three rural villages in Teluk Datuk area, some 45 miles from Kuala Lumpur showed that worm infestations were common. About 95% of the school children, aged 6-12 years were infested with one or more worms (Lo *et al,* 1979). Breakdown of the results is as given in Table 10.

Lower infestation rates were reported by George and Ow-Yang (1982) for some 7,000 urban school children from 143 primary schools and 49 secondary schools in the Kuala Lumpur Federal Territory; the overall prevalence rate was 50.1%. Infestation rates according to age was found to be as given in Table 11. It was also found that infestation rates in Malays (67%) and Indians (64%) were higher than that in the Chinese (33%).

Table 10. Helminthic Infestation in Rural Villages in Teluk Datuk

Sex of Subjects	no. of subjects examined	one or more worms	prevalence rate (%)			infestation (%)		
			ascaris	trichuris	hookworm	single	double	triple
male	435	95.2	86.4	84.1	48.1	13.3	40.2	41.6
female	399	94.7	87.0	85.0	37.8	12.0	50.4	32.3
both	834	95.0	86.7	84.5	43.2	12.7	45.1	37.2
average worm load (eggs per millitre)	834	—	59,700	6,233	1,964			

Source: Lo *et al*, 1979

Table 11. Intestinal Helminths in Urban School Children of Kuala Lumpur

age	no of children examined	prevalence rate, %			
		overall infestation rate	ascaris	trichuris	hookworm
6-7 years	2,576	48.2	23.8	42.9	1.6
11-12 years	2,450	57.3	27.1	51.9	4.6
14-15 years	1,706	47.0	16.8	41.0	6.7
16-17 years	950	42.6	12.3	35.7	9.3
combined	7,682	50.1	21.9	44.5	4.6

Source: George and Ow-Yang, 1982

5.5.1 Effects of Helminthic Infestations on Anemia

Although prevalence of intestinal parasitic infestation of anemic subjects has been fairly well studied, few workers had investigated into the effects of these infestations on anemia. Most of the work in this area were carried out by Tasker of the Institute for Medical Research in the late 1950s and 60s (Tasker, 1958a, 1961).

A detailed study of the association between worm infestation and anemia was first reported by Tasker in 1958a. Upon examination of the hemoglobin and parasite ova for 1,400 patients in the General Hospital, Kuala Lumpur, an association as seen in Figure 5 was obtained:

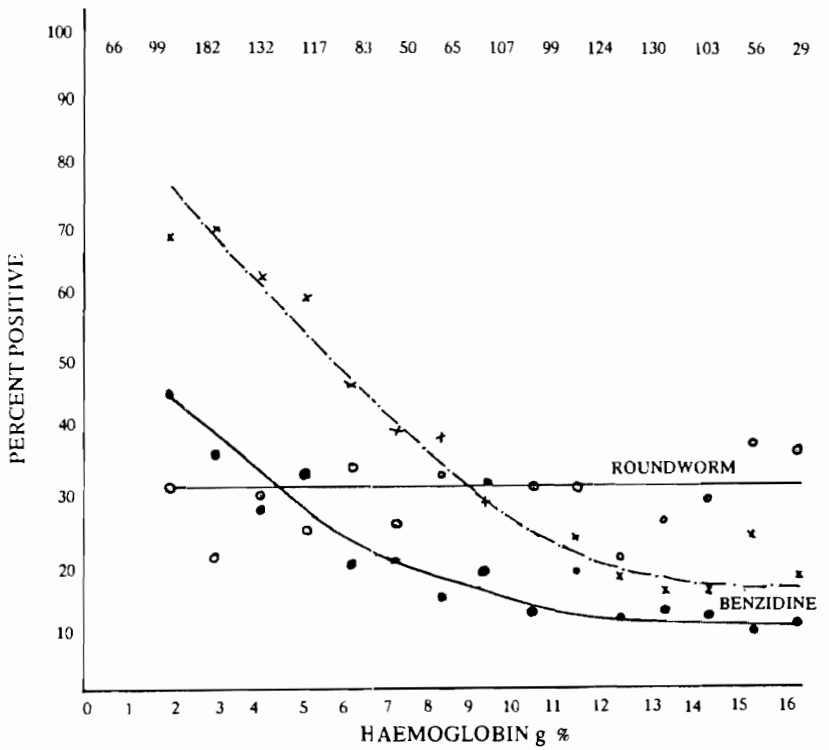


Figure 5. Relationship Between Hemoglobin Levels and the Results of the Stool Examinations for Hookworm and Roundworm Ova and the Benzidine Test.

Figures at the top of graph indicate the number of patients studied in each hemoglobin group. Source: Tasker, 1958a

It was found that the lower the hemoglobin levels in these patients, the higher was the proportion found to have hookworm ova in their stools. The percentage of stools showing a positive benzidine test (for blood in stool) also increased with advancing anemia. In contrast, the proportion of stools containing roundworm ova *(Ascaris lumbricoides)* was unrelated to the degree of anemia.

Further analysis of the results revealed that in patients with megaloblastic anemia, there was no association between severity of anemia and hookworm infestation. This contrasted markedly with the association found in the patients suffering from iron deficiency anemia (Tasker, 1958a) (see Fig.6 Below).

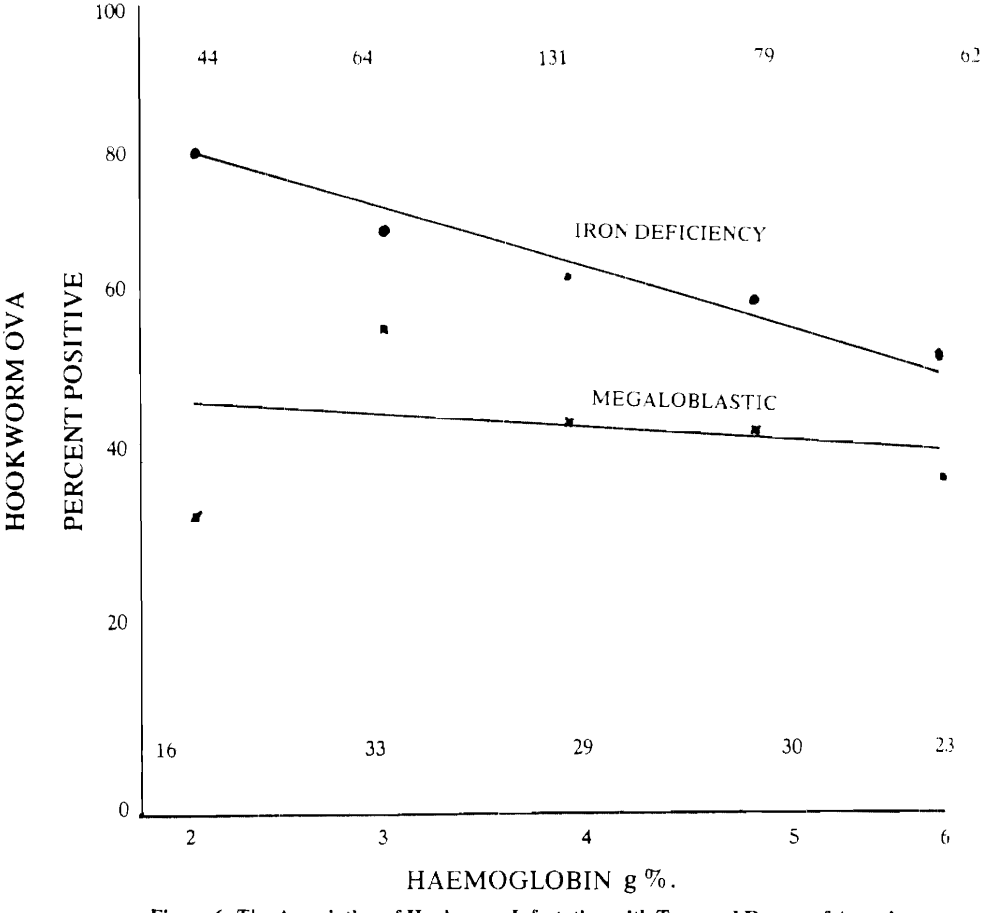


Figure 6. The Association of Hookworm Infestation with Type and Degree of Anemia.
Figures at the top and bottom of graph indicate the number of patients studied
Source: Tasker, 1958a

The results were said to suggest that hookworm infestation was partially responsible for the iron deficiency anemia observed in these subjects. A study of the association between albumin: globulin ratio of serum proteins and hookworm infestation rate did not give a significant correlation. It was concluded that malnutrition did not appear to increase the likelihood of a hookworm infestation (Tasker, 1958a).

Using the technique of radioactive tracer, where red blood cells were labelled with ^{51} Cr, Tasker (1961) studied blood loss as a result of hookworm infestation in 20 anemic subjects (hemoglobin 3-10 g/dl). The association between intestinal blood loss and hookworm load (all worms recovered from the stool were identified as *N. americanus)* is shown in Figure 7.

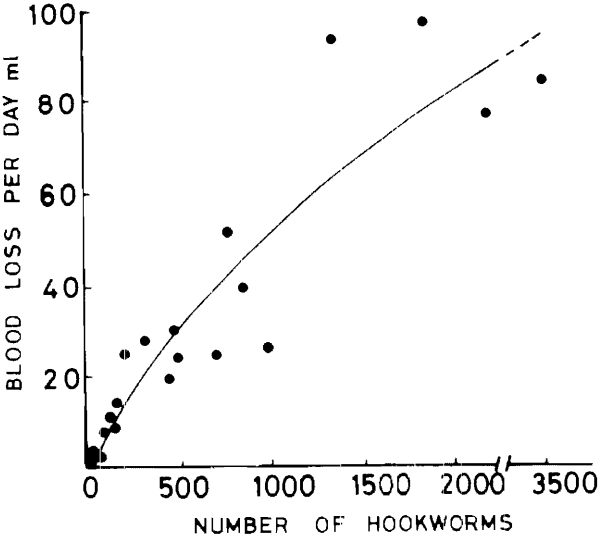


Figure 7. Relationship between Intestinal Blood Loss and Hookworm Load
Source: Tasker, 1961

Daily blood loss in these subjects was found to increase from about 2 ml with a light infestation of about 20 worms to about 90 ml with a heavy infestation of greater than 1,500 worms. Hence, even a light worm load of about 100 worms would have caused a daily blood loss of about 8 ml. At normal hemoglobin level of 14 g/dl, it was estimated

that the daily iron loss could be about 4 mg. On a marginal dietary intake of iron, such a loss could result in a negative iron balance. The patient would become anemic and as a result, the iron loss would decrease. Assuming a constant iron intake, worm load and blood loss, a new balance could be reached at a lower hemoglobin level. However, heavy infestations would easily deplete a patient of iron, even with a good dietary iron intake, and anemia would then be progressive and severe (Tasker, 1961).

More recently, Chong *et al* (1982) reported an association between intestinal helminthic infestation and growth. It was observed that in the rural Malay children studied, the infested children were more physically retarded than those who were worm-free (see Table 12).

Table 12
Interaction of Helminthic Infestations and Nutrition in Preschool Children

Nutritional index (expressed as Mean ± S.D.)	not infested (n=50)	infested (n=97)
Wt-for-age as % of reference	87.3 ± 12.2	81.4 ± 10.4 [b]
Ht-for-age as % of reference	94.1 ± 5.4	92.3 ± 4.6 [a]
Wt-for-Ht as % of reference	97.3 ± 8.9	93.6 ± 6.7 [b]
MAC as % of reference	90.5 ± 6.8	89.3 ± 7.4

[a] Significantly different at $p < 0.05$, [b] Significantly different at $p < 0.01$
Source: Chong *et al* (1982)

6 LABORATORY DIAGNOSIS

There has been continuous refinement and improvement in laboratory methods for the diagnosis of nutritional anemia. New methods have also been introduced. A wide spectrum of methodologies are now available to assist the clinician in the hospital or the nutritionist in the field in the identification as well as the monitor of the anemia condition during therapy. As has been pointed out by Cook (1982), there is no single test or combination of tests that is optimal in all clinical settings. For instance, the optimal set of methods is different for the infant, pregnant women and the elderly patient. Similarly, the detection of mild iron deficiency would require a different approach than that for the hospitilized patient with severe anemia. The World Health Organization had often emphasized the need for standardization of procedures if meaningful comparisons were to be made between results of surveys in different parts of the world (WHO, 1968; WHO, 1972). To this end, a standard methodology for all WHO sponsored or coordinated investigations was established and "reference centres" were set up, which helped develop methods and established quality control among collaborating laboratories (Baker and DeMaeyer, 1979).

6.1 DIAGNOSIS OF IRON DEFICIENCY ANEMIA

Traditionally, iron deficiency has been considered synonymous with microcytic hypochromic anemia. Examination of peripheral blood smear was once thought to be the simplest and most direct approach to diagnosing iron deficiency anemia. It has however been emphasized that morphologic changes in erythrocytes are both very nonspecific and very insensitive as criteria of iron deficiency (Jacobs, 1974; Fairbanks, 1978). It is now clear that a firm diagnosis requires additional laboratory measurements. A battery of methods are now available, each with its own merits and demerits. Some of the methods to be described below have been described in detail in a comprehensive review of iron methodology edited by Cook (1980) and should prove a useful reference manual for laboratories.

As discussed earlier (Section 5.2), iron lack may be divided into 3 stages, depending on the severity of the deficiency. This discussion of the methodologies for the assessing of iron status will be made in line with each of these stages. The sensitivity of various iron measurements varies with this severity. Suggested key laboratory measurements for each stage are as tabulated below:-

Deficiency stage		Laboratory investigation
Storage Iron Depletion (Pre-latent)	1.	hepatic iron content
	2.	phlebotomy
	3.	iron absorption studies
	4.	serum ferritin
	5.	marrow iron
Iron Deficiency Erythropoiesis (Latent)	1.	transferrin saturation
	2.	free erythrocyte protoporphyrin
	3.	erythrocyte indices
Iron Deficiency Anemia (Manifest Iron Deficiency)	1.	hemoglobin or hematocrit
	2.	therapeutic trial

6.1.1 Indicators of Iron Storage

Several investigatory approaches have been used to determine the body iron stores. Although most of these procedures find little application in field studies, they may provide a yardstick against which simpler methodologies can be measured. Bothwell *et al* (1979), Torrance and Bothwell (1980) and Worwood (1980) have described in detail the various approaches available.

6.1.1.1 Hepatic iron content

Baker and DeMaeyer (1979) described a WHO collaborative international study to determine the relative size of iron stores by the examination of liver specimens obtained at autopsy. Results of these studies will be described under Section 7 on "Prevalence".

6.1.1.2 Bone marrow iron

This technique is said to be the time-honored method for assessing storage iron. Since the reticuloendothelial cells of the marrow represent an appreciable part of the storage compartments for iron, and since the bone marrow is the most accessible site for examining these cells, histologic evaluation of marrow iron could provide a direct measure of iron status (Fairbanks, Fahey and Beutler, 1971; Bothwell *et al*, 1979). The evaluation of marrow iron has been used extensively and considered one of the simplest ways for hospitalized patients. There is however recent evidence that the examination of marrow iron may not be as infallible as generally believed. Disparity of results has been reported between using aspirated and needle-biopsy specimens of marrow (Fong, Okafor and Thomas, 1977; Krause, Brubaker and Kaplan, 1979).

6.1.1.3 Phlebotomy

The only strictly quantitative method for measuring body iron is to repeatedly phlebotomize the subject, usually in 500 ml units, at weekly intervals until significant anemia develops (Cook, 1982). By determining the total amount of hemoglobin iron removed and the induced deficit in circulating hemoglobin iron, the iron stores originally present can then be calculated by subtraction. Summarising results obtained by various investigators using this method, Bothwell *et al* (1979) reported a range of 600-1000 mg of storage iron from Western adult males and only about 200-300 mg in females. Little information is said to be available from other parts of the world, but the study of Sood, Banerji and Ramalingaswami (1968) suggested that the mobilizable iron stores were lower than 600 mg.

6.1.1.4 Absorption of radioactively labelled iron

Heinrich (1970) had discussed at length the practical applications of an accurate and quantitative determination of intestinal iron absorption, such as by the ^{59}Fe-absorption whole body retention test in the diagnosis of prelatent iron deficiency, its frequency, patho-physiology and treatment. According to Finch (1970), this is the most quantitative method for the measurement of storage iron, since iron stores are the most important factor in determining the absorptive behaviour of the intestinal mucosa. It was further suggested that with a 5 mg dose of ferrous ascorbate, an absorption of over 20% will probably occur with iron stores of less than 400 mg; with iron stores of over 800 mg, absorption would usually be less than 10%. It has been suggested that where a whole body counter is available, this is one way of studying the distribution of iron stores in the community (Baker and DeMaeyer, 1979).

6.1.1.5 Serum ferritin

Body iron in excess of that required for hemoglobin formation has been known to be stored in tissues as ferritin. This soluble protein is normally present in such small quantities in serum (10-200 ng/ml) (Jacobs *et al,* 1972) that it had remained undetected. It is only recently that Addison *et al* (1972) first demonstrated, using a sensitive immunoradiometric assay, that tiny quantities of ferritin were invariably present in human sera and that the concentration of the protein varied with body iron stores. Following this report, numerous investigators studied into the relationship between serum ferritin and iron status of individuals. Reviewing the evidences put forth, Cook (1982) concluds that in normal subjects, there is ample evidence that serum ferritin is directly proportional to body iron stores. The most convincing evidence is said to have come from correlation studies in which iron stores were measured quantitatively by phlebotomy (such as the report by Walters,

Miller and Worwood, 1973) or directly by iron absorption (e.g. Cook, Lipschitz and Miles, 1974). Summarising from various studies, Cook (1982) reported that within the range of 20-300 μg/l, 1 μg/l serum ferritin would be equivalent to 10 mg storage iron.

Serum ferritin is now a commonly used tool for anemia studies. At all ages, a serum ferritin value below 10 or 12 μg/l indicates depletion of iron reserves (corresponding to about 100 mg iron stores, deemed to be the least severe stage of iron depletion) (Cook and Finch, 1979; Dallman, Siimes and Stekel, 1980; Bothwell and Charlton, 1981). It is regarded by some to be the most sensitive parameter of iron status (Alfrey, 1978; Cook and Finch, 1979), and said (Cook, 1980) to be the most important advance in iron methodology in the past decade.

Serum ferritin measurement is said to have several advantages over other methods. It is able to distinguish between true iron deficiency and the anemia of chronic inflammation (Lipschitz, Cook and Finch, 1974), is relatively stable with repeated measurements in the same subject (Pilon *et al*, 1981), and it allows evaluation of iron nutrition within the normal range, as well as in conditions of iron deficiency and excess (Siimes, Addiego and Dallman, 1974; Jacobs and Worwood, 1975; Lipschitz, Cook and Finch, 1974). However it is to be noted that ferritin measurement does not give a sharp distinction between normal and iron deficient subjects (Fairbanks, 1978).

Serum ferritin measurements could be technically relatively difficult due to the complex methodologies. If employing the immunoradiometric assay (IRMA) using labelled antibody, and radioimmunoassays (RIA) using labelled antigens, a gamma counter is essential (a review of these two radioisotope assay methods has been given by Alfrey, 1978). Recently, the use of enzyme-linked immunoassay (ELISA) method which eliminates the need for radioisotopes has been reported (e.g Anderson and Kelly, 1981). There is an increasing number of commercial ferritin assay kits in the market. As expected, these are relatively costly.

6.1.2 Measurements of Iron Deficient erythropoiesis

6.1.2.1 Transferrin saturation

When iron stores are depleted, the serum iron transport parameters, serum iron, total iron binding capacity (TIBC) and transferrin saturation change: TIBC would increase whilst serum iron declines; hence transferrin saturation also decreases. For general purposes, Bainton and Finch (1964) felt that the most useful parameter of iron supply to the developing red cell is the percentage of transferrin saturation (calculated by dividing the concentration of serum iron by the TIBC and multiplying by 100 to express the

result as a percentage) rather than the plasma iron *per se*. These investigators suggest that a decrease below 16% is to be taken as indicative of iron deficiency. Koerper and Dallman (1977) and Saarinen and Siimes (1977) have suggested that lower limits (between 7 and 10%) be used for infants and adolescents. This has now become a commonly used parameter in screening iron deficiency, and is said to be able to detect twice as many iron deficient individuals as can hemoglobin determinations (Cook and Finch, 1979). Dallman, Siimes and Stekel (1980) suggested that for survey purposes, it is essential that the assay be used in combination with other tests and that the lower normal values in infants and children should be taken into account in the interpretation. Details of the procedures are described in Fielding (1980) and Bothwell *et al* (1979).

The method, however, can be quite time consuming if done manually and subject to error from extraneous iron contamination. Precision is also said to be limited by wide diurnal variations in normal individuals, and a relatively mild or transient infection can bring about a fall in transferrin saturation values and hence not specific for iron deficiency (Cook and Finch, 1979, Cook, 1982).

6.1.2.2 Free erythrocyte protoporphyrin (FEP)

Since protoporphyrin is the complex that combines with iron to form hemoglobin, a lack of iron supply to the developing red cell would impair heme synthesis and result in an accumulation of protoporphyrin IX in the circulating red cell. The introduction of simple and rapid techniques for measuring FEP has brought about another important laboratory parameter of iron deficient erythropoiesis. Labbe and Finch (1980) has described in detail a procedure for FEP determination.

Although providing about the same information as transferrin saturation, FEP is said to be more stable. Whilst transferrin saturation can change within a matter of hours, FEP increases only a week after iron supplies become deficient and returns to normal only slowly after treatment of the iron deficiency (Langer *et al*, 1972). Normal FEP has been reported as 35 $\pm$ 50 μg/dl RBC and levels greater than 100 μg/dl have been suggested to indicate overt iron deficient erythropoiesis (Cook, 1982). Recently, it has been suggested that expressing results as FEP: hemoglobin ratios (μg protoporphyrin/g hemoglobin) could be a useful clinical parameter (Piomelli, Brickman and Carlos, 1976; Thomas *et al*, (1977). The mean protoporphyrin: hemoglobin ratio has been reported to be 16.0 $\pm$ 3 and an upper cut-off level of 32 has been suggested (Cook, 1982). Another recent development is the introduction of hematofluorometers that claims to permit measurement of FEP directly in a drop of unprocessed blood on a glass slide (Blumberg *et al*, 1977).

One of the problems associated with the use of FEP is the well recognized fact that the level is also increased in lead poisoning. A careful inquiry into possible lead exposure, measurement of blood lead levels, and demonstration of a fall in FEP with iron therapy could serve to exclude lead poisoning (Thomas *et al*, 1977; Cook, 1982).

6.1.2.3 Erythrocyte indices

Another measurement for the latent stage of iron deficiency is the red cell indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). In terms of sensitivity, red cell indices are said to be intermediate between measurements of iron deficiency and frank iron deficiency anemia. Hypochromic and microcytic red cells will appear in the circulation before a significant decrease in hemoglobin concentration has occurred (Cook, 1982).

There appears to be some controversy regarding the usefulness of each erythrocyte index in the diagnosis of iron deficiency. MCV determinations used to be derived from the ratio of the hematocrit to the red cell count obtained manually using a microscope. The introduction of electronic counting equipments enables this. The parameter to be measured directly, thereby making this once time-consuming and poorly reproducibe test an accurate and practical laboratory procedure (Dallman, Siimes and Stekel, 1980). It is also said to be highly reproducible and less subjected to sampling error than, for example, hemoglobin determinations. Dilution by tissue fluid or fluctuations in plasma volume also do not affect red cell size. Hence the MCV, done electronically, is believed to be more sensitive than the other erythrocyte indices (Fairbanks, 1980). However, low values of MCV are also found in thalassemias (which are quite common in some areas including Southeast Asian countries) and in chronic disorders such as infections, inflammation and malignancy (Dallman, Siimes and Stekel, 1980; Fairbanks 1980). Some MCV values for all age groups are tabulated in Dallman, Siimes and Stekel (1980) based on results reported in Dallman and Siimes (1979).

The other two red cell indices can also be obtained by electronic counters but they are not directly measured like the MCV. The MCH is derived by dividing hemoglobin concentration by red cell count and has lesser diagnostic value. MCHC, the least directly measured (actually could be calculated by dividing hemoglobin by hematocrit) is also the least useful of the indices (Cook 1982). On the other hand, Bothwell *et al* (1979) feel that MCH is the most accurate determination in uncomplicated anemia, since both red cell count and hemoglobin are directly and accurately measured.

In evaluating these indices obtained from electronic cell counters, Klee *et al* (1976) concluded that none of these erythrocyte measurements were sufficiently accurate to be used for routine diagnosis of iron deficiency without the use

additional laboratory tests. However, they felt that these indices could be useful in screening patients so as to determine which patients should have additional laboratory tests.

Whatever has been said regarding these indices will not be applicable if counting of cells is done manually, as is the case in the hospital laboratories in Malaysia. Electronic counters are currently used only in institutions in Kuala Lumpur and the various Universities in the country. Under the circumstances, both the MCV and the MCH cannot be very reproducible; the best index in terms of reproducibility would be the MCHC, which is derived from the hemoglobin concentration and the hematocrit, both of which are reasonably accurately measured by manual means.

6.1.3 Manifest Iron Deficiency Anemia

6.1.3.1 Hemoglobin

The final stage of iron deficiency is associated with a significant decrease in circulating hemoglobin. Measurement of hemoglobin concentration of venous or capillary blood specimens has probably been one of the commonest methods of determining prevalence of anemia in many community surveys. Since iron deficiency is, in all populations, by far the commonest cause of anemia, the frequency of anemia thus obtained has served as a useful index of iron deficiency. It is however clear that other causes of anemia are also common in many populations, such as thalassemias and hemoglobinopathies, chronic infectious diseases and other nutritional disorders e.g. folate and vitamin B_{12} deficiency (Fairbanks, 1978). The main limitation of hemoglobin as a measure of iron deficiency is the marked overlap in frequency distribution curves in anemic and normal individuals (discussed earlier in Section 2). Hence, use of this criterion alone for determining anemia would result in a large number of false negative and false positive results (Cook *et al*, 1971).

Another frequently noted problem regarding the use of hemoglobin as a parameter is in deciding what constitutes an abnormal hemoglobin concentration. The question of what cut-off values to use would be of importance especially in prevalence studies in communities. The criteria for anemia suggested by the WHO (1968) that has become widely accepted in recent years (Cook, 1982) are as shown in Table 13. Since the levels recommended are for residents at sea level, surveys of population groups at high altitudes would necessarily need to develop a different set of criteria (Fairbanks, 1978). However, Garn, Smith and Olark (1975a,b), Dallman *et al* (1978) and Dallman (1981) had pointed out that the American blacks had a consistently lower hemoglobin than the whites, indicating a possible need for a lower limit for normal hemoglobin levels in the former group. Hence Baker (1981) feels

that it can no longer be taken for granted that the frequency distribution of normal hemoglobin concentration in the different races of Asia will be the same as in Caucasians and there is an urgent need to determine this frequency distribution in each demographic sector of the various ethnic groups in the Asian region.

Table 13
Hemoglobin Levels Below Which Anemia is likely to be present in Populations living at sea-level

Children, 6 months to 6 years	11 g/dl
Children, 6 - 14 years	12
Adult males	13
Adult females, non-pregnant	12
Adult females, pregnant	11

Source WHO, 1968

Furthermore, with regard to the cut-off level for pregnant women, some investigators feel that the level should be set at 10 g/dl (and corresponding 0.30 for hematocrit value) especially for the tropics. Harrison (1982), for example, feels that this may be justified because in many developing countries, the vast majority of women with hemoglobin levels of around 10 g/dl are apparently healthy and symptom-free and perinatal mortality rates are no different from what they are at higher hemoglobin levels.

The concentration of hemoglobin is usually measured after dilution of the blood sample in Drabkin's solution to convert the hemoglobin to cyanmethemoglobin, which is then quantitated using a spectrophotometer. A simple but much less reproducible method involves using a visual colour comparator.

6.1.3.2 Hematocrit

Hematocrit and hemoglobin values have often been regarded as important and inexpensive methods for screening subjects in field studies. The hematocrit is technically simple to perform. A minute amount of blood is collected in a heparinised capillary tube and centrifuged in a micro-hematocrit centrifuge. Hematocrit is then calculated by comparing the height of the column of packed red cells with the height of the entire column of red cells and plasma. General nutritional and medical practice have assumed that the hematocrit and hemoglobin are equally useful in detecting anemia and that they can be used inter-changeably. When mean values in large groups of subjects are compared, the hematocrit is generally equivalent to the hemoglobin multiplied by 3 (Dallman, Siimes and Stekel, 1980). However, recent evidences presented seem to suggest that this assumption requires re-

examination. Graitcer, Goldsby and Nichaman (1981) analysing the results of studies of 13,040 children concluded that hemoglobin and hematocrit screening tests are indeed not comparable in detecting anemia in the same population. Using only hematocrit tests, anemia was diagnosed in 1 to 10% of children with normal hemoglobin levels and was not detected in 20-50% of children who might be considered anemic on the basis of low hemoglobin levels. These investigators however emphasize that their report does not suggest that the hematocrit test is of no value as a screening test, but merely points out that its use should take into consideration its difficult reproducibility, and its incomparability with hemoglobin cut-off values.

6.1.3.3 Therapeutic trial

The serum ferritin is to the detection of storage iron depletion what the therapeutic iron trial is to the detection of iron deficiency anemia. They are the most sensitive measures of early and late iron deficiency respectively and they also provide precise quantitative information on the deficit in body iron (Cook, 1982). Some workers feel that the most conclusive evidence of iron deficiency anemia is a significant rise in hemoglobin and/or PCV following oral or parenteral iron therapy (Graby, Irnell and Werner 1969a, b; Dallman, Siimes and Stekel, 1980; Cook, 1982). The therapeutic trial has been said to be helpful in detecting subclinical iron deficiency in prevalence studies and in determining the relative importance of iron lack when the cause of anemia is multifactorial. Cook (1982) had suggested that an increase of 2 g/dl of hemoglobin the the tiral would be more reliable although an increase of 1 g/dl is often considered significant. The method would clearly be of limited practical usefulness for population screenings and because of the time required to make a diagnosis, would not be helpful for hospitalized patients. Moreover, if placebo groups are considered, ethical considerations are becoming more of a constraint on using these groups.

6.1.4 Methodologies for Population Surveys: General Considerations

It is clear from the above discussion that there is no single parameter that monitors the entire spectrum of iron status. The distinction of iron deficiency into 3 stages is in fact arbitary since iron status in a population undoubtedly forms a continuum ranging from storage iron depletion to frank iron deficiency anemia. Hence, because of the dynamic nature of iron metabolism, measurements taken at one point and at one time must be viewed cautiously (Cavill, 1982).

It is to be expected that the combined use of two or more independent indicators would greatly improve diagnostic accuracy. Hemoglobin alone, as discussed earlier, cannot be used to identify iron deficiency because other

factors such as folate deficiency or chronic infection may cause anemia. Transferrin saturation, FEP, and serum ferritin have therefore often been suggested to be included in assessing iron status of a population. Various investigators have emphasized the usefulness of the combined use of various parameters (Cook, Finch and Smith, 1976; Thomas *et al,* 1977; Cook and Finch, 1979; Hershko *et al,* 1981). There appear to be some differences in opinion amongst these investigators as to which combination would be the most suitable for use. As suggested by Dallman, Siimes and Stekel (1980), the "most suitable combination" would depend on various factors such as the prevalence of iron deficiency, and whether co-existing complicating conditions (e.g. infection, thalassemia, or lead poisoning) are prevalent in the population being studied. Another important factor to be considered is the volume of blood sample that can be obtained from the subjects. This could be rather problematical when dealing with infants and young children. Obviously, the selection of tests for the prevalence study would also depend on the availability of manpower and equipment to carry out the tests.

Cavill (1982) felt that a practical definition of iron deficiency should include both anemia and reduced reticulo-endothelial stores. Hence a hemoglobin concentration of less than 12 g/dl with a serum ferritin concentration of less than 12 µg/l was suggested to be a good indication of iron deficiency which warrants iron therapy. It was however also pointed out that this simple definition could be complicated in those chronic or malignant diseases in which marrow activity is suppressed. Cavill (1982) in his review of the diagnostic methods for iron deficiency has also pointed out that there is no point trying to define iron deficiency by looking at interrelationship of several factors and measuring the degree of concordance of one with another. It was felt that the value of any one parameter would only depend upon the arbitrary choice of the true measure against which the others are tested. It was emphasized that it is necessary to consider the significance of each individual parameter and to interpret the results accordingly.

6.2 DIAGNOSIS OF FOLIC ACID AND VITAMIN B_{12} DEFICIENCIES

6.2.1 Peripheral Blood and Bone Marrow Examination

Examination of peripheral blood and bone marrow is probably the simplest means of the diagnosis of megaloblastic anemia, resulting from deficiency of folic acid and/or vitamin B_{12}. The earliest detectable change in the peripheral blood may be nuclear hypersegmentation in the polymorphs. Subsequently, characteristic changes are macrocytosis, anisocytosis and poikilocytosis of the red cells, neutropenia and thrombocytopenia (Baker, 1967). Changes in the marrow vary according to the severity and duration of the deficiency. The classical morphological hall-marks of these deficiencies are the changes in the developing red cells. In the severest forms, the marrow will be markedly hypercellular and will contain many large primitive megaloblasts. In lesser degrees of deficiency, abnormalities ranging from the most severe to the just abnormal may be seen. The ultimate test of abnormality is to observe a change towards normality in the morphology of the nucleated red cells following specific vitamin treatment. These changes of megaloblastosis may, however be masked by severe iron deficiency, by defects of hemoglobin synthesis and by the presence of B_{12} deficiency in the presence of adequate amounts of folic acid. There may also be changes in the white cells in the marrow, such as increase in size of these cells, characteristic giant metamyelocytes, giant band or "stab" cells and large hypersegmented polymorphs or "macropolycytes" (Baker, 1966).

There is however a considerable degree of morphological and biochemical similarities at the cellular level, as well as clinical and therapeutic overlap, between changes due to folate deficiency and those due to vitamin B_{12} deficiency (Das and Herbert, 1976). Hence, these morphological changes are not absolutely diagnostic of vitamin B_{12} or folate deficiency. The advent of assay methods for measuring the concentration of these vitamins has provided a more sensitive method for ascertaining the diagnosis (Baker, 1966). Furthermore, once the diagnosis of megaloblastic anemia has been established by pheripheral blood and marrow examination, it is necessary to differentiate between deficiency of folate and vitamin B_{12}. Sullivan (1970) has particularly discussed and emphasized this differential diagnosis of megaloblastic anemia.

6.2.2 Folic Acid and Vitamin B_{12} Assay in Biological Fluids

Folic acid may be estimated by chemical, biological and microbiological methods. The chemical methods are generally suitable only for pharmaceutical preparations, since their sensitivity is inadequate to detect the low folate concentrations occurring in biological materials (Malin, 1975). Biological estimations using chicks and rats have been developed, but they are relatively more expen-

sive and require a longer time (Malin, 1975). The most useful methods for the assay of small amounts of folate in biological materials have proved to be microbiological assay methods (Stokstad and Koch, 1967; Chanarin, 1979). Microbiological assays, however, are complicated by the existence of inactive conjugates and a large number of naturally occurring metabolic forms of folic acid possessing varying activities for different assay organisms (Stokstad and Koch, 1967). Malin (1975) and Hoffbrand (1978) have pointed out the availability of more recently developed methods of serum and red-cell folate determination such as isotope dilution techniques using a binding protein derived from milk and radioactively labelled folic acid or methyltetrahydrofolate. The principle of these radioassay methods have been reviewed by Rothenberg and Da Costa (1976). These assays are said to be advantageous over the microbiological assay methods in that they may be performed in a relatively short time and, perhaps more importantly, because they are not affected by drugs which have been found to inhibit the growth of *L. casei*. However, such methods would be out of the reach of many laboratories

Laboratory diagnosis of folate deficiency in man has usually been made by determining serum and red cell folate levels. For the measurement of serum folate levels, the organism used is usually *Lactobacillus casei*. This organism, as mentioned erlier, responds to a number of folate forms including N^5 methyltetrahydrofolate, which is the chief folate form in serum (Baker, 1966; Stokstad and Koch, 1967). On the other hand, the folate in the red cell is largely, if not entirely, in the form of a polyglutamate which would be unavailable for the growth of *L. casei;* a pre-treatment with conjugase is therefore necessary (Stokstad and Koch, 1967; Chanarin 1979). Methods used in WHO collaborative studies (Baker and DeMaeyer, 1979) are those described by Baker *et al* (1959) who were also the first to introduce the use of serum folate assay (Chanarin, 1979) with further details given in Herbert *et al* (1960) and Herbert (1966). A satisfactory technique (Chanarin, 1979) for preparation of red cells for microbiological assay has been described by Hoffbrand, Newcombe and Mollin (1966).

Mean levels of serum and red cell folate of "normal" subjects reported by various research groups have been summarised by Chanarin (1979). Mean serum folate values ranged from 5.1 to 9.9 ng/ml and mean red cell folate concentrations ranged from 144 to 454 ng/ml. The difficulty of defining a normal range was pointed out and it was emphasized that each laboratory should have its own normal range. It was however suggested that as a generalization, healthy subjects have a lower value of 3 ng/ml and an upper limit of 25 ng/ml for serum folate; the lower limit of red cell folate in normal subjects was thought to be 100 ng/ml, with an upper limit of 400 to 800 ng/ml. The use of these cut-off values, <3 ng/ml serum folate and <100 ng/ml red cell folate, has also been described by Masawe (1981).

Although the normal range is established, interpretation of the results of serum assays is not always straightforward. Baker (1966) and Hoffbrand (1978) have pointed out the following point when interpreting serum and red cell folate values. A lowered serum folate level is an accurate guide to the presence of folate deficiency. However, it is to be noted that: (a) falsely low results may be obtained due to inhibition of the microbiological assay by drugs in the serum; (b) equally low results may be obtained in patients with extremely severe deficiency; and (c) non-deficiency subjects whose intake of folate has fallen only for a few days may also give low results. Normal serum folate levels do not always rule out folate deficiency: they may be normal or raised in vitamin B_{12} deficiency due to accumulation of improperly utilized N^5 methyltetrahydrofolate in the plasma, while the overall body stores of folate are depleted.

It is generally felt that red cell folate is a better guide than serum folate to tissue folate stores. A subnormal level normally means folate deficiency of some standing and severity. However, in vitamin B_{12} deficiency, red cell folate concentrations are also known to fall since uptake of folate by erythrocyte precursors requires adequate cobalamin function, so that a low level alone does not distinguish the two deficiencies when present together. Thus Hoffbrand (1978) has suggested that the assay of red cell folate be used in conjunction with serum B_{12} and folate determination.

The monitor of urinary excretion of formiminoglutamic acid (FIGLU) has also been used as a test for folate deficiency. It differs in principle from the other methods used in that it does not depend on measuring levels of the vitamin, but on assessing an enzymatic function in which the vitamin participates. In deficiency states, formiminoglutamic acid, an intermediary in the catabolism of histidine would acculumate and be excreted in abnormally large amounts in the urine following an oral dose of histidine. Details of this are given in Chanarin (1979).

As with serum folate, serum vitamin B_{12} has most commonly been estimated by microbiological assay methods. The organisms most frequently used are *Lactobacillus leichmannii* and *Euglena gracilis* (Baker, 1966; Chanarin, 1979). Detailed discussion of the methods are given in Chanarin (1979). According to Chanarin (1976), measurement of serum B_{12} levels remains the most useful of the laboratory tests used in diagnosis for all practical purposes, it is always lowered in B_{12} deficiency. The use of radioisotope dilution assay methods for B_{12} has been reviewed by Mollin, Anderson and Burman (1976). Another test which is specific for diagnosis of vitamin B_{12} is increased methylmalonic acid excretion following the administration of valine (details are given in Chanarin, 1979). The measurement of methylmalonic acid, however, has not yet been sufficiently simplified for routine clinical application (Rottenberg and Cotter, 1978).

According to Rottenberg and Cotter (1978), the finding of a serum vitamin B_{12} concentration of between 150 and 200 pg/ml by radioassay or between 100 and 150 pg/ml by microbiological assay is suggestive of vitamin B_{12} deficiency. The authors however pointed out that exceptions to this are: (a) in pregnancy, where there is a more rapid transfer of vitamin B_{12} to the fetal circulation, thus lowering the concentration of the vitamin in maternal blood; (b) in early malabsorption, where deficient absorption of B_{12} may lower serum level by 20-30% before tissue stores are actually depleted. They have suggested that when serum B_{12} concentration is below 150 pg/ml by radioassay or below 100 pg/ml by microbiological assay, vitamin B_{12} deficiency is most likely.

The other test which has been described for the diagnosis of megaloblastic anemia is the deoxyuridine suppression test (Chanarin, 1976; Rottenberg and Cotter 1978). This is thought to be an indirect measure of thymidylate synthetase activity. The test is abnormal in megaloblatic anemia due to either vitamin B_{12} or folate deficiency and correlates with the severity of the morphological changes. Differential correction of the suppression test can be used to distinguish vitamin B_{12} from folate deficiency. In therapeutic trials (Baker, 1967; Rothenberg and Cotter, 1978), frequent measurements of reticulocyte response and hemoglobin are made following therapy with a physiological dose of vitamin B_{12} (1-2 µg) and/or folic acid (50-200 µg). This is said to be most valuable in analysing the cause of megaloblastic anemia in patients with low serum levels of both vitamin B_{12} or folate. The method is however too tedious for routine use.

7 PREVALENCE

7.1 ANEMIA : A GLOBAL PROBLEM

As "chlorosis", iron deficiency anemia was considered epidemic in Europe and in the European Settlements of the western hemisphere more than a century ago; only near the turn of this century did it become clear that chlorosis was, in fact, iron deficiency anemia (Fairbanks, 1978). The earliest epidemiologic study of nutritional anemia was perhaps that of Mackay (1931), who in the years 1925 to 1930, surveyed anemia in 1100 infants and 168 expectant or nursing mothers in London, England (Fairbanks, 1978). Subsequently, the problem was extensively surveyed in many parts of the "western" countries. More recently, numerous studies have also been carried out in different parts of Asia. Clearly, the prevalence varies from one country to another, and in the same country, from one place to another and in the same place, amongst different community groups. There have also been indications that it even varies with the time of the year (Fleming, 1970; Foster, 1968; Masawe, 1981). However, it is clear that the results of the different studies are not strictly comparable, since the methodologies employed were often not standardized, or even unacceptable (Baker, 1981), and as Masawe (1981) added, different criteria for defining anemia have been used. Furthermore, it was felt that the majority of the studies have been carried out in selected population samples which may not have been representative of the population as a whole (Baker, 1981). Be that as it may, the various studies are probably a good indicator of the probable prevalence in the communities at large, and as Baker (1981) admitted, they could reflect the situation in the general population.

The anemia problem had long been recognized by the World Health Organization (WHO, 1959) as "a public health problem of considerable importance in the underdeveloped and tropical areas of the world", and it has repeatedly emphasized the high prevalence of the problem (WHO, 1968, 1972, 1975). It has been further noted that there has been no lessening in the prevalence and severity of anemia in the tropics during the past two to three decades (Woodruff, 1982).

It has been adequately shown that of the three major hemopoietic nutrients, iron deficiency is the most common cause of anemia (WHO, 1959, 1968, 1975; Baker, 1978, 1981; Masawe, 1981; Woodruff, 1982). There is undoubtedly a widespread and high prevalence of iron deficiency in the world (Baker and Mathan, 1975; Fairbanks, 1978; Bothwell *et al*, 1979; Royston, 1982). It is in fact thought by some to be the most commonly recognized form of nutritional deficiency in developing countries as well as in affluent countries (Beaton, 1974; Dallman, Siimes and Stekel, 1980). It has been estimated that there are more than 500 million people throughout the world with iron deficiency, and perhaps 20 million in the United States (Finch and Huebers, 1982).

Until the 1960s there was little information about the prevalence of megaloblastic anemias resulting from folate and/or vitamin B_{12} deficiencies, although it was realized that such deficiencies did occur in substantial numbers (WHO, 1968). It was however soon clear that next to iron deficiency, anemia due to folate deficiency was the most prevalent (WHO, 1975), especially amongst pregnant women (Baker, 1978). Vitamin B_{12} deficiencies are known to be relatively rare (WHO, 1975; Baker, 1978).

As discussed earlier, there are times in the life of the individual when hemopoietic nutrient balance is particularly precarious, such as during the rapid growth period of the young child, and during the child-bearing age of a woman, especially during pregnancy and lactation. These have been aptly called the vulnerable groups, and studies have shown that prevalence of nutritional anemia is particularly high amongst these groups. Harrison (1982) estimates that from 5 to 50% of all pregnant women attending antenatal clinics in the tropics are found to be anemic, compared with under 2% in the developed countries. It is however known that in certain parts of Asia, even adult males, who are usually least at risk, show a high prevalence of anemia (Baker, 1978); this would be particularly so amongst the lower-socio-economic segments of the communities.

It would not be at all possible to review even a portion of the studies that have been carried out to determine the prevalence of anemia around the world. Only recent major studies or reviews of such studies will be highlighted below. Studies carried out in this country will however be presented in some detail.

Since pregnant women are at greater risk, the prevalence of anemia in these women has frequently been studied and used as an indicator of the prevalence of anemia of the population at large (Baker, 1981). The various collaborative WHO studies on women in the 3rd trimester of pregnancy in different countries have recently been collated and reviewed by Baker and DeMaeyer (1979). The studies were undertaken in Israel, Poland (Warsaw), Northern India (New Delhi), Mexico, Venezuela (Caracas) (these were first reported in WHO (1968)), Southern Indian (first briefly reported in WHO (1968); reported in full by Yusufji, Mathan and Baker (1973)), seven Latin American countries (reported by Cook *et al,* 1971) and Burma. The women studied were generally hospital or clinic populations, and were mostly from the lower socio-economic strata of society. Standardized methods of diagnosis were used and common references or cut-off values were employed. The results reported are here summarised and re-tabulated in Table 14.

A moderate to very high prevalence of anemia (as indicated by low hemoglobin values) amongst pregnant women in the communities studied was reported; the highest prevalence appeared to have been found in India and Burma. In all cases, prevalence rates were higher in pregnancy than the

Table 14

Prevalence of Anemia, Iron, Folate and Vitamin B_{12} Deficiencies in Collaborative Studies of Women in the Third Trimester of Pregnancy, Compared with non-Pregnant Women and Men

Geographical area	% anemic [a]	% with iron deficiency (transferrin saturation <15%)	% with folate deficiency (serum folate <3 ng/ml)	% with B_{12} deficiency (serum B_{12} <80 pg/ml)
Warsaw, Poland:	21.8	40.0	1.4	0.5
Latin America.	26.5	49.0	11.1	24.3
Mexico:				
pregnant women	26.6	61.2	6.5	7.1
non-pregnant women	11.7	28.1	6.0	0
men	0.9	3.6	3.5	1.1
Venezuela:				
pregnant women	37.0	59.7	15.1	23.0
non-pregnant	14.9	18.9	9.5	1.0
men	1.9	0	18.8	2.0
Israel:				
pregnant women	47.0	46.3	6.3	2.0
non-pregnant women	29.0	11.4	5.1	0
men	13.6	8.8	1.6	0
Southern India, (Vellore):				
pregnant women	56.0	99.0	9.0	0.
non-pregnant women	35.0	42.5	0	3.0
men	6.0	5.1	2.0	0
Northern India (Delhi):				
pregnant women	80.0	51.7	–	49.0
non-pregnant women	64.3	25.8	–	26.7
Burma:	82.0		46.0	0

* based on the following hemoglobin cut-off values:

pregnant women	<11 g/dl
non-pregnant women	<12 g/dl
men	<13 g/dl

except Mexico where values increased by 1 g/dl for all groups.

Sources: WHO, (1968); Yusufji, Mathan and Baker, (1973); Cook *et al* (1971) and Baker and DeMayer (1979).

non-pregnant controls, who in turn showed a higher prevalence than the men, in agreement with the well established fact that the pregnant women are the most at risk to anemia. This is also true for all the other indices tabulated. Prevalence of iron deficiency anemia, as indicated by a low transferrin saturation value, was even higher than that for anemia in all the areas studied (with the possible exception of Israel) in line with the commonly held view that in countries where there is a high prevalence of anemia, there would be an even higher prevalence of iron deficiency (Baker, 1981). In Poland, only 1% of the pregnant women had a serum folate value below 3

ng/ml; in all other countries, about 10% of them had serum folate below this level, whilst the Burmese study showed the highest prevalence of 46%. If the cut-off was set at 6 ng/ml, pregnant women in all the countries studied had a prevalence of low serum folate of about 50%, with the exception of Poland, with only 16%, and Burma, with 92% (Baker and DeMayer, 1979). In some of the countries, there was also evidence of vitamin B_{12} deficiency; a fairly high prevalence of low serum vitamin B_{12} levels was found in the Latin American, Venezuelan and North Indian studies.

The other major WHO collaborative studies reviewed by Baker and DeMaeyer (1979) were the determination of the size of iron stores by the examination of liver specimens obtained at autopsy. Over 3000 post-mortem liver specimens were obtained in duplicate, either from subjects dying from trauma, or those dying in hospital from a variety of conditions. These were forwarded to two different centres, one for the histochemical quantitation of hemosiderin content and the other for chemical estimation of non-heme iron. For the former, sections of the liver were made, stained for hemosiderin, and the amount of stainable iron graded on a score of 0 to 5, where 0 indicates absence of stainable iron. The mean of the grade for all the specimens for the population group was then calculated (details given in Banerji, Sood and Ramalingaswami, 1968). The chemical analysis of the non-heme iron content of the specimens was performed in the laboratory of Charlton *et al* (1970). Results summarised by Baker and DeMaeyer (1979) are reproduced in Tables 15 and 16.

Table 15

Grading of Hemosiderin in Livers of Male and Female Subjects of Different Countries Collected in Collaborative WHO Studies

countries/ communities	mean score	% subjects with no iron
South African Indians	0.41	80
Mexico	0.45	68
India	0.53	67
Venezuela	0.69	58
United Kingdom	0.78	53
United States	0.80	44
Venezuela*	0.94	–
Sweden*	1.03	–
Czechoslovakia*	1.08	–
South African Whites	1.46	26
South African Bantus	2.61	18

*Data from hospital autopsies; all other figures based on medicolegal autopsies from acute accidents deaths.

Source: Banerji *et al* (1968); tabulated in Baker and DeMeyer, 1979).

As pointed out by Baker and DeMaeyer (1979), although these specimens were not necessarily representative of the population from which they were obtained, they nevertheless underline the fact that iron deficiency, as shown by low iron stores, is not confined to developing countries. It is found in a considerable proportion of the population, particularly in women of reproductive age, even in countries like the United Kingdom and the United States.

Table 16
Nonheme Iron Content of Livers Collected in Collaborative WHO Studies from Subjects of Different Countries

countries/ communities	ug iron/g tissue males	females
India (Delhi)	93	68
New Guinea	106	80
United Kingdom	111	117
Columbia	117	84
Sweden	159	129
United States (Seattle)	173	103
Venezuela (White)	177	108
Mexico	196	136
Czechoslovakia	212	182
South Africa (White)	258	161
South Africa (Bantu)	776	268

Source: Charlton *et al* (1970); tabulated in Baker and DeMayer (1979).

Fairbanks (1979) has compiled an extensive review of the epidemiologic surveys of anemia and iron deficiency carried out from 1967 onwards. A total of some 80 reports were included in the review (including the 8 studies discussed above), covering many different countries of the world. Few data were however accesible for Soviet Union, China, Pakistan and the Antilles. Various age groups were included, covering mostly infants, young children and pregnant women, as well as adult men and non-pregnant women. There was no uniformity in the criteria used for determining anemia and iron deficiency; one really cannot expect there to be one. Summarising the results, Fairbanks (1978) concluded that:

1. iron deficiency was widely prevalent in all population groups studied, constituting a worldwide problem of extraordinary magnitude;
2. it is particularly prevalent in pre-school children of economically deprived families irrespective of race, and in women of child-bearing age and in pregnancy; and
3. prevalence rates were higher in all ages and both sexes in tropical areas, due both to the frequency of intestinal infestation (especially hookworm) and to consumption of a "meat-poor" diet, common in many communities of tropical areas.

Masawe (1981) has recently reviewed the prevalence of nutritional anemia in Africa. Studies were done in various parts of the continent, in the 1960s and 70s (results were not tabulated). Two series were considered: community-based studies and hospital-based studies. In the former group of studies, mild to moderate rates of prevalence were reported (subjects for the studies were mostly rural adults). Iron deficiency was found to be the commonest cause and tended to occur mostly in hot, damp area. In one area

(Natal), over 40% of the males were found to be anemic (hemoglobin <13 g/dl). Masawe (1981) pointed out the interesting finding of the study of the Banthus in Durban, where the climate is sub-tropical and iron deficiency anemia was found to be rare. It is to be noted that the criterion for anemia (using hemoglobin concentration) was not uniform for all the studies. In the hospital-based group of studies, Masawe (1981) noted that nutritional anemia was a very common cause of admissions to medical wards, in pediatric wards and obstetric services. Iron deficiency appears to be the major cause of anemia; prevalence of megaloblastic anemia tends to be low. Masawe also noted the seasonal variations of the prevalence of anemia, the frequency tending to increase during the latter half of the dry season, and at the beginning of the wet season, probably because food supplies, especially green vegetables, tended to be scarce during these times. A slightly different approach in discussing the anemia problem in Africa has been taken by Fleming (1982) in his review.

A recent review of the prevalence of anemia in Asia by Baker (1981) is also of interest. Except for a few studies in the 1960s, most of the 16 studies reviewed were carried out in the 70s and two studies in 1980. Most of these studies were not covered in the review of Fairbanks (1978) (discussed above). Baker (1981) felt that the results of the studies indicate a high prevalence of anemia throughout tropical Asia in most sectors of the population.

Royston (1982), from the Division of Family Health, WHO, has recently reviewed the prevalence of nutritional anemia in women in developing countries. It is estimated that nearly 500 million women are living in developing countries (other than China), out of which 70 million of them are pregnant and at least as many lactating. From the information collated, Royston (1982) concluded that about half of the non-pregnant women and nearly two-thirds of the pregnant women could be considered "anemic", giving a total of some 230 million "anemic" women in these countries. The overall proportion of anemic women appears to be the highest in Asia and Oceania, followed in descending order of magnitude by Africa and Latin America. For pregnant women, the order is said to be the same. Reported data for each of the countries surveyed were tabulated. Wherever information was available, mean serum iron, folate and vitamin B_{12} were also tabulated. The population (women between 15 to 49 years) at risk, number and estimated percentage of women with hemoglobin concentrations below the norm were also tabulated for the countries.

7.2 PREVALENCE OF ANEMIA IN MALAYSIA

None of the published reviews so far discussed have touched on the prevalence of anemia in Malaysia in depth. Some of the studies cited include those by Tasker (1958b), ICNND (1963), Lourdenadin (1964), Llewellyn-Jones (1965), Chappel and Janowitz (1965), Chong *et al* (1968), Sinhu (1974) and Ong (1974). Various recent studies, especially those by the Institute for Medical Research have not been cited. Furthermore, there has been no comprehensive review of the anemia problem in the country. Hence an attempt will be made to review all reported studies of the prevalence of anemia in the country. Older studies will be mentioned, but more recent studies will be dealt with in some detail. There has been a considerable amount of re-tabulation and collation of results of various studies so as to present a clearer picture of the problem. Results have also been re-grouped into those dealing with children and pregnant women, the recognized vulnerable groups, as well as those dealing with labourers and industrial workers, due to recent emphasis on anemia and work productivity.

7.2.1 Anemia in Malaysian Children

There have been few studies devoted specially to studying the problem of anemia in children in the country. Various nutrition surveys conducted had however placed emphasis on examining this segment of the populatin. All of the studies reviewed were community based studies, with the exception of the study of Lie-Ijo and Virik (1966). This will be dealt with first.

Hemoglobin was determined in 2,025 children, of whom 851 were Chinese, 734 Indian and 440 Malays admitted to the children's ward of the General Hospital, Kuala Lumpur (Lie-Injo and Virik, 1966). Results presented showed that the lowest hemoglobin levels occured in children aged 6-36 months and that the Indian children were the worst off. The hemoglobin level of 258 children (13%) were 8 g/dl or less, many of whom were admitted to the hospital for diseases other than anemia. Upon further examination of 179 of these children with very low hemoglobin, 108 (60.3%) of them were found to be suffering from iron-deficiency anemia and 20 (11.2%) of them had megaloblastic changes in the bone marrow due to folic acid and vitamin B_{12} deficiencies. Hence more than 70% of the anemia encountered were of nutritional origin.

The study reported by Bourne (1949) immediately after the Japanese occupation of Malaya was probably one of the earliest large scale nutrition survey in the country. Though not specifically directed towards the problem of anemia in children, the survey included hemoglobin determinations of various groups of children. 730 children attending welfare centres, 224 orphans, and 287 children in a refugee camp were amongst the groups studied. Very bad cases of malnutrition were said to be evident amongst these children, especially in the refugee camp. Percentages of children with

hemoglobin levels below 70% (Talquist scale) were 71% 41.7% and 90% respectively for the three groups mentioned.

The survey also included a study of 3,627 school children attending English-medium schools in Selangor. These children came from more well-to-do classes of the community and were expected to be one of the nutritionally better-off groups. In spite of this, it was reported that there was an appreciable amount of malnutrition and their greatest deficiency was in the hemoglobin concentration; 40.3% of the boys and 62.9% of the girls had values below 70%. Examination of 3,580 children in what were called 'vernacular' schools at that time showed that they were nutritionally slightly worse off than those in English Schools. Hemoglobin levels were very low; approximately 40% of both boys and girls had hemoglobin values of less than 70%.

One of the earliest studies devoted specifically to children was that of Wardsworth and Lee in 1959. A simple hemoglobin detemination was carried out in 261 boys and 174 girls with ages 7-15 years in 6 different schools in the district of Muar, Johore. These were a mixed group of Malay and Chinese children, all of whom were from the lower income group. It was found that a considerable amount of moderate anemia existed among these children. The authors emphasized that such degrees of anemia should not be regarded as unimportant, "as they may lead to deleterious effects in the course of time, and could account for loss of physiological efficiency and possibly for a number of symptoms of ill-health".

Other general nutrition surveys in the country had also placed emphasis on children in their study and hemoglobin determinations were commonly done. In more recent studies, methods and cut-off values were more standardised (the cyanmethemoglobin method was used, unless otherwise specified). Other measurements such as micro-hematocrit, serum iron and TIBC, or a combination of these were reported in some of these studies. Findings on anemia from the more recent major studies are summarised in Tables 17 to 25, and figure 8.

Based only on the hemoglobin values and using a cut-off of 12 g/dl for all age groups, the ICNND (1964) study (Table 17) reported that 36% of children less than 5 years of age and 13% of age group 5-14 years were anemic. The condition was thought to be due to a number of different causes, including protein deficiency, inadequate dietary supplies of iron, infestation with parasites and possibly a small number due to sprue or other forms of malabsorption syndromes.

The study of Chappel and Janowitz (1965) used the criterion of 10 g/dl hemoglobin for anemia. Table 18 shows that the distribution of anemia was highest amongst the younger children ($\leq$ 6 years) and appear to be highest for the 1 - 2 years age group. Dietary iron deficiency was thought to be the main causative factor.

Detailed findings from the study of Chong (1970) in Telok Datok, Kuala Langat are given in Table 19. These findings were compared with those obtained for a group of children from Ulu Trengganu and some army children in the report of Chong, McKay and Lim (1972) (Table 20). This group of children from the East Coast state of Trengganu are seen to be worst off in hematocrit values.

In the study reported by Amir Abbas (1973), a total of 425 pre-school children (ages between 6 months to 7 years) in Kampung Selisek, Ulu Selangor district were examined. A "varying degree of malnutrition" was observed. Histogram (Fig. 8) shows that the percentage of anemic pre-school children in all the villages studied ranged from about 50% to 70%. The average for the whole area was reported to be 62.1%. Only 62% of these children were found to have malaria parasite in their blood film.

Table 17. Anemia Amongst Boys and Girls of Civilians and Military Dependents (ICNND, 1964)

		both sexes	boys		girls	
		<5 yrs	5-9yrs	10-14yrs	5-9yrs	10-14yrs
(a)	Hemoglobin					
	n	59	55	80	60	7
	mean (g/dl)	12.1	12.5	13.3	13.2	13.7
	S.E	–	0.33	0.13	0.18	0.17
	freq. distribution (%):					
	<12.0 ("deficient")	35.6	18.2	10.0	16.7	8.4
	12.0-13.9 ("low")	59.3	58.2	65.0	53.3	52.1
	14.0-14.9 ("acceptable")	3.4	21.8	20.0	25.0	22.5
	>15.0 ("high")	1.7	1.8	5.0	5.0	16.9
(b)	Hematocrit (PCV):					
	n	60	55	78	59	66
	mean (%)	35.3	37.6	39.7	39.5	39.7
	S.E.	–	0.66	0.32	0.34	0.45
	freq distribution (%):					
	<36 ("deficient")	50	18.2	6.4	8.5	10.6
	36-41 ("low")	48.3	69.1	70.5	69.5	59.1
	42-44 ("acceptable")	0	10.9	7.9	16.9	19.7
	>45 ("high")	1.7	1.8	5.1	5.1	10.6
(c)	MCHC:					
	n	52	55	78	59	66
	mean (%)	33.8	33.0	33.6	33.4	34.6
	S.E.	–	0.51	0.32	0.38	0.64
	freq. distribution (%)					
	<28.0	3.8	9.1	3.8	3.4	3.0
	28.0-29.9	7.7	3.6	2.6	6.8	1.5
	30.0-31.9	7.7	7.3	12.8	22.0	16.7
	>32.0	80.8	80.0	80.8	67.8	78.8

Table 18
Anemia in Rural Malay Children and Adolescents in FLDA*, Sungai Tekam, Pahang

age (years)	male n	male mean Hb(g/dl)	male % anemic (<10 g/dl Hb)	female n	female mean Hb(g/dl)	female % anemic (< 10 g/dl Hb)
0 - 1	17	9.7	53	12	9.9	58
1 - 2	19	9.3	69	22	9.9	59
3 - 6	56	10.4	34	23	10.1	35
7 - 10	30	10.9	13	28	11.3	21
11 - 20	20	11.3	20	26	11.3	19
combined	142	10.4	–	111	10.6	–

*FLDA = Federal Land Development Authority
Source: Chappel and Janowitz, 1965.

Table 19. Anemia Amongst Pre-School Children in ANP Pilot Area, Telok Datok, Kuala Langat

age gp (months)	n	hemoglobin mean, g/dl	hemoglobin % < 11 g/dl	PCV mean	PCV % < 32%
0 - 12	34	11.7	–	36	–
13 - 24	27	11.8	–	36	–
25 - 36	32	12.2	–	38	–
37 - 48	34	12.9	–	38	–
49 - 60	5	11.0	–	34	–
combined	132	–	27	–	11

Source: Chong, 1970.

Table 20. Comparison of Hematocrit Value of Malay Pre-school Children from Three Communities

	Ulu Trengganu	Army	Kuala Langat
n	203	127	128
mean PCV, %	32	36	37
S.D.	5.0	3.0	3.5
range	14 - 45 %	27 - 43 %	23 - 45 %
% with PCV < 32 %	37	11	11

Source: Chong, McKay and Lim, 1972.

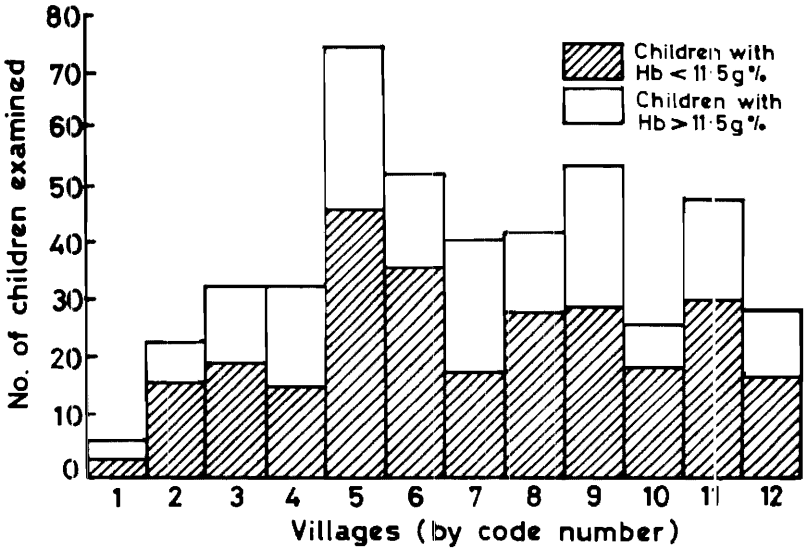


Figure 8. Anemia Amongst Pre-school Children in Village of Ulu Selangor District
Source: Amir Abbas, 1973

The study of N. Kandiah and co-workers of children in two Malay communities and an Indian estate population is summarised in Table 21. Most of the anemic cases were thought to be due to iron deficiency as shown by red cell changes in the peripheral blood film. Only a few cases of abnormal hemoglobin were found and infection rate of malaria parasites amongst these children was reported to be very low (this was not studied for the Indian estate community). The authors pointed out that the prevalence rate of anemia for pre-school children in Ulu Jempol was the highest reported during the time. It is however to be noted that in the series studied by Amir Abbas (1973), almost all the villages had a prevalence of over 50% and overall prevalence for the 12 villages was 62.1%

Table 21

Prevalence of Anemia Amongst Children in Two Malay Communities and an Indian Estate Population

		pre-school children	school children	
(a)	Rural Malays, Ulu Jempol, Negeri Sembilan (Kandiah & Lim, 1976)			
	n	72 (1-6yrs)	486 (7-14yrs)	70 (15-19yrs)
	% anemic	51.0	18.6	10.0
(b)	Malays, SLDA* Scheme, Ulu Rening, Selangor (Kandiah & Tan, 1977)			
	n	39 (1-6yrs)	73 (7-17yrs)	
	% anemic	23.1	11.0	
(c)	Indians, rubber estate, Selangor (Kandiah & Lim, 1977)			
	n	57 (0-7yrs)	247 (8-18yrs)	
	% anemic	47.4	37.2	

*SLDA = State Land Development Authority

Criteria for Anemia:

pre-school children, Hb $<$ 11.0 g/dl
school children, Hb $<$ 12.0 g/dl

Several studies of A.J.U. Anderson on children of various communities in Sarawak are compiled in Table 22. The highest prevalence of anemia was observed for the 1 year-olds and the infants for all the communities studied (except for the Land Dayaks of the Tebakang area, for which age breakdown of prevalence was not available). This was thought to be due to indequate supplementary feeding and poor weaning practices, with lack of iron, protein and other nutrients. Intestinal helminths were said to be a contributory causal factor in the anemias encountered.

Table 22
Prevalence of Anemia Amongst Children of Various Communities in Sarawak

Communities Studied	age groups									
	6-11 mths	1 yr	2yrs	3yrs	4yrs	5yrs	6yrs	7yrs	8yrs	all ages
a. Land Dayak, Tebakang (Anderson, 1976a)										
n	–	–	–	–	–	–	–	–	–	667
% anemic	–	–	–	–	–	–	–	–	–	27.7
b. Iban, Middle Mukah River (Anderson, 1976b)										
n	51*	64	59	66	64	69	70	51	51	545
% anemic	31.3	35.9	33.8	12.1	10.9	7.2	20.0	13.7	3.9	18.7
c. Iban, Lemanak River (Anderson, 1977a)										
n	32	59	61	51	51	53	54	53	59	478
mean Hb, g/dl	10.6	10.3	11.2	11.4	11.5	11.8	12.0	12.0	12.0	11.5
% anemic	59.4	57.6	23.0	21.6	27.5	11.3	51.9	39.7	32.2	35.1
i. Iban, Sut & Mujong Rivers (Anderson, 1978a)										
n	24	67	67	68	58	55	46	47	41	473
mean Hb, g/dl	10.2	10.4	11.6	11.9	12.0	12.2	12.3	12.0	12.2	11.7
% anemic	50.0	58.2	26.9	14.7	15.5	7.3	28.3	44.7	36.6	29.8
. Malays, Sarawak River Delta (Anderson, 1977b)										
n	32	44	43	48	41	48	68	68	87	479
% anemic	40.6	52.3	23.3	29.2	19.5	16.7	50.0	36.8	32.2	34.0
Penan, Gunong Mulu (Anderson, 1978b)										
n	24	67	67	68	58	55	46	47	41	473
mean Hb, g/dl	10.2	10.4	11.6	11.9	12.0	12.2	12.3	12.0	12.2	11.7
% anemia	50.0	58.2	26,9	14.7	15.5	7.3	28.3	44.7	36.6	29.8

*= 0 - 11 months
Criteria for anemia:
6 mths - 5 yrs, Hb <11.0 g/dl
6 yrs - 8 yrs, Hb < 12.0 g/dl

Prevalence of anemia, based on hemoglobin values, reported by Chen *et al* (1981) of children of various ethnic groups in the Interior, West Coast and Kudat Divisions of Sabah is tabulated in Table 23. MCHC values were also reported; based on the prevalence of children with MCHC value of $<31\%$, it was suggested that about 15% of them were likely to suffer from iron deficiency.

Table 23
Prevalence of Anemia
Amongst Children of Various Ethnic Groups
in the Interior, West Coast and Kudat Divisions, Sabah

age (years)	percent anemic		
	male	female	combined
0.5 - 2	34.5	27.4	30.9
2 - 4	14.8	26.4	20.2
4 - 6	32.4	19.1	25.0
6 - 8	28.1	31.7	29.8
8 - 10	29.6	27.4	28.5
10 - 12	22.8	23.3	23.0
12 - 13	17.6	13.5	15.8
0.5 - 13	26.4	25.6	26.0

Study population: 0 - 4 yrs = 795
5 - 12 yrs = 2877
total n = 3672

Criteria for anemia: 6 mths - 5 yrs, Hb $<$ 11 g/dl
6 yrs - 14 yrs, Hb $<$ 12 g/dl

Source: Chen *et al*, 1981

Recent studies of Chong and co-workers of the IMR on various Malay rural poverty communities in different states of the Peninsular Malaysia are compiled in Table 24. The anemias encountered were again said to be primarily due to iron deficiency.

Table 24
Recent Data on the Prevalence of Anemia Amongst Rural Malay Children

communities studies	infants	pre-school children	primary school children	
a. Kuala Trengganu, Trengganu (Chong, 1974)				
n	—	399	--	
% anemic:				
Hb < 11.0g/dl	—	23	--	
PCV < 33%	—	9	--	
b. Kota Baru, Kelantan (Chong *et al*, 1979)				
n	—	64	174	
% anemic	—	33	73 (boys) 56 (girls)	
c. Mersing, Johore (Chong *et al*, 1981)				
n	—	123	146	
Hb, mean ± SD, g/dl	—	12.3 ± 1.5	13.3 ± 1.4	
% anemic	—	16	16	
d. Baling, Kedah (Chong *et al*, 1982)				
n	30	243	122 (boys)	120 (girls)
Hb, mean ± SD, g/dl	10.3 ± 1.3	11.2 ± 1.4	11.9 ± 1.8	12.5 ± 1.6
% anemic	83	41	45	37
MCHC, mean ± SD, %	32.9 ± 5.5	30.8 ± 3.2	31.5 ± 3.8	31.9 ± 3.1
% anemic	36	53	40	38
e. Perak Tengah, Perak (Chong *et al*, 1983)				
n	24	187	283	
Hb, mean ± SD, g/dl	10.9 ± 1.1	11.4 ± 1.7	12.6 ± 1.5	
% anemic	12	32	27	

Criteria for anemia: <6 yrs, HB < 11 g/dl
6 - 14 yrs, Hb < 12 g/dl
and MCHC < 31%

Another recent study by the IMR was a survey of three malaria endemic villages of Bengkoka Peninsula, Sabah. The anemia encountered (Kandiah *et al*, 1983) was said to be related to iron deficiency, because a high proportion of them were with "low" MCHC values. It was noted that anemia was observed as early as infancy and amongst the very young children and that no age groups were spared. Malaria was also believed to play a role in the anemia; the malaria parasitemia rate of these children was estimated to be 24%. Results of this survey are tabulated in Table 25.

Table 25
Anemia Amongst Pre-school Children
in Three Malaria Endemic Villages of Bengkoka Peninsula, Sabah

age groups (months)	hemoglobin mean ± S.D (g/dl)	hemoglobin % children <11 g/dl	MCHC mean ± S.D. (%)	MCHC % children <31%	PCV mean ± S.D (%)
< 12	10.7 ± 1.6	67	31 ± 2.7	50	36 ± 2.3
12 - 23	10.5 ± 1.9	53	30 ± 2.7	67	36 ± 2.3
24 - 35	11.1 ± 1.3	36	32 ± 2.1	55	36 ± 3.1
36 - 47	11.4 ± 0.8	53	31 ± 1.7	50	36 ± 2
48 - 59	11.4 ± 1.6	38	32 ± 2.5	31	36 ± 3.2
60 - 72	11.8 ± 0.9	18	33 ± 2.4	22	37 ± .9
all ages (n = 90)	11.1 ± 1.5	44	31 ± 2.7	48 ± 2.9	3.6 ± 2.9

Source: Kandiah *et al*, 1983

These studies reviewed have shown that there is a mild to moderate prevalence of anemia in the communities studied, ranging from 10 to 50%, with most areas having 20 - 30% prevalence rate. The condition in Peninsular Malaysia is no better than those areas studied in Sarawak and Sabah. Anemia was said by various investigators to be mostly of the iron deficiency type. Some research groups noted a higher prevalence amongst the 12 - 36 month old children.

7.2.2 Anemia And Pregnancy in Malaysia

Early investigators in the country had recognized that anemia was a major cause of maternal mortality, especially during pregnancy (Reed, 1940). Tasker, Richardson and Llewellyn-Jones (1956) pointed out that the incidence of anemia amongst pregnant women was high and that they appeared to be the most susceptible group of the population. Anemia was said to be one of the main complications of pregnancy (Tasker, 1956). According to this investigator, about 5% of those delivered in the General Hospital, Kuala Lumpur had hemoglobin levels lower than 6.5 g/dl and usually about one in eight of the available ante-natal beds were in use for the treatment of anemia (Tasker, 1958b). The magnitude of the problem then could be reflected from the words of an obstetrician actively involved in this field: "the problem of anemia in pregnancy is still the nightmare of practising obstetricians in this country" (Lourdenadin, 1964).

One of the earliest reports in the country on the prevalence of anemia amongst pregnant women was that of Corke and Bush in 1930, in which results of a small series of 26 Indian pregnant women was reported. Another report in the 1930s on the subject was that of Pallister (1934). In the discus-

sion of Reed (1940) on the problem of "maternal mortality among South India estate women", it was emphasized that anemia was "the great cause of death" amongst these women. These early reports had emphasized the importance of nutrient deficiencies in the causation of anemia and that pregnancy aggravated the condition.

The report of Tasker, Richardson and Llewellyn-Jones (1956) described the screening of 5500 antenatal subjects in the obstetrical unit of the General Hospital, Kuala Lumpur for low hemoglobin values in 1954. A total of 296 of these, or 5.3%, were admitted for detailed hematological studies and treatment of anemia. The incidence rate of admission for anemia in 1953 was said to be 6.3%. In a later report, Tasker, one of the most active researchers in anemia at the time, reviewed his 5 years experience in dealing with the problem of anemia in pregnancy in the obstetrical unit of the Hospital (Tasker, 1958b).The review covered aspects such as the prevalence of anemia in pregnancy, its management and documented the effects (fetal loss and prematurity) upon pregnancy. Tasker had emphasized, in both these two reports, that iron deficiency was almost invariably present in patients with nutritional anemia. Upon this basic deficiency, additional types of anemia, of which that associated with a megaloblastic marrow were said to be the most important and common.

The problem of anemia during pregnancy continued to draw the attention of nutritionists and obstetricians in the country in the 1960s. Wadsworth and Lee (1959) described their experiences with over 200 subjects in Johore. Lourdenadin (1964), Llewllyn-Jones (1965), Chong *et al* (1968)) and Lourdenadin (1969) continued to highlight the problem in the Kuala Lumpur area. The data reported by some of these investigators are summarised below to give some idea of the prevalence of anemia amongst expectant Malaysian women at the time.

Lourdenadin (1964) summarised the prevalence of severe anemia as seen in the Maternity Hospital, Kuala Lumpur in a retrospective study for the period 1957 - 1961. The results are reproduced in Table 26.

Table 26. Prevalence of Severe Anemia in the Maternity Hospital, Kuala Lumpur from 1957 - 1961

	hemoglobin levels (gr/dl)		
year	0.0 – 2.49	2.5 – 4.9	5.0 – 6.5
1957	2	83	124
1958	1	86	113
1959	1	86	218
1960	5	62	150
1961	3	45	78
no. of patients	12	362	683
percentage	1.1	33.9	65.0

total no. of anemia cases ($<$ 6.5 g dl) = 1066
total no. of deliveries = 46912
incidence of severe anemia = 2.2%
Source: Lourdenadin, 1964

The types of anemia encountered were as shown in Table 27. It was observed that microcytic anemia, said to be caused by "defective nutrition and iron deficiency", formed about 76% of the total number of anemic patients. The remaining was mostly macrocytic anemia, with folic acid and vitamin B_{12} deficiencies implicated (Lourdenadin, 1964).

Table 27. Types of Anemia Ecountered in the Maternity Hospital, Kuala Lumpur from 1957 - 1961

	1957	1958	1959	total	percentage
Microcytic hypochromic	139	148	265	552	76.2
Di-morphic Anemia	1	1	5	7	1.0
Macrocytic Anemia	69	52	39	160	22.1
Hemolytic Anemia	3	1	1	5	0.7
Total cases	212	202	310	724	100.0

Source: Lourdenadin, 1964

In the same report (Lourdenadin, 1964), the results of a prospective study of the hemoglobin levels of a thousand consecutive pregnant mothers attending the ante-natal clinic of the Hospital were also reported, results of which are retabulated in Table 28. A total of 76.9% of these women studied were found to be anemic.

Table 28. Hemoglobin Values of Expectant Mothers at Maternity Hospital, Kuala Lumpur (Lourdenadin, 1964)

	mothers at clinic studied		
race	n	% of total	average Hb (% Haldane scale)*
Chinese	400	37.2	65.7
Indians	360	33.5	65.6
Malays	128	11.9	66.4
Others	188	17.4	78.7
all races	1076	100	68.7

*A hemoglobin of 76% Haldane scale or 11 g/dl was taken to be the lower limit of normality.
Source: Lourdenadin, 1964

Similar figures were reported by Llewellyn-Jones (1965) for the same hospital, but for a longer time period, between 1953 and 1962. A total of 73,048 women were seen at the Maternity Hospital during the period, and as shown in Table 29, 2250 or 3.1% had severe anemia (Hb $<$ 6.5 g/dl) and the majority of this is of the iron deficiency type.

Table 29. Anemias Amongst Ante-natal Subjects of the Maternity Hospital, Kuala Lumpur (1953 - 1962)

	no. with the following Hb (g/dl)			
type of anemias	0-2.49	2.5-4.99	5.0-6.5	total
normoblastic iron deficiency	16	216	444	676 (63%)[a]
megaloblastic	12	215	146	373 (35%)
hemolytic	2	7	8	17 (2%)
total diagnosed	30	438	598	1066
unknown[b]	12	380	792	1184
Grand total	42	818	1390	2250

[a]Percent of total cases with types of anemia diagnosed
[b]Unknown = accurate diagnosis was not made
Source: Llewellyn-Jones, 1965

Some hematological data for a series of 98 women attending the Maternity Hospital, Kuala Lumpur and a nearby Municipal Health Clinic for antenatal care were reported by Chong *et al* (1968) (retabulated in Table 30). Based on a hemoglobin value of $<$ 10 g/dl, 11% of the subjects were said to be anemic. These invetigators had emphasized the importance of detecting anemia early and treating the affected adequately. The roles of various fac-

tors in the development of anemia were extensively discussed. Malnutrition, especially with regards to protein, iron, folic acid and vitamin B_{12} deficiencies, malaria and parasitic infestations were recognized as important etiological factors. The hazards of anemia in pregnancy, labour and the puerperium, and the effects on the fetus were also discussed.

Table 30
Some Hematological Data of Expectant Women
(Chong *et al.* 1968)

	mean ± S.D.
n	98
hemoglobin, g/dl	12.0 ± 1.3
PCV, %	36.0 ± 3.4
RBC Counts, 10^6 /mm^3	4.11 ± 0.66
MCV, u^3	85.0 ± 8.5
MCH, μg	28.0 ± 3.6
MCHC, %	33.1 ± 2.1
Peripheral Blood Film (% with mild to moderate hyprochromia)	16.0

Source: Chong *et al.* 1968

Few studies into the nutritional anemia problem during pregnancy were reported in the 1970s. One such study however stands out. Ong (1973) reported hematological data of 278 pregnant Orang Asli women at the Orang Asli Hospital, Gombak, Kuala Lumpur. The results (summarised in Table 31) were said to suggest a mild degree of iron deficiency anemia in the average pregnant Orang Asli women. Differences in hematological values between "deep" jungle and "outside" jungle populations were briefly discussed. It was thought that this difference could be due to migration of the latter group to settle down near villages or towns, which caused changes in their life style, having to earn money to buy food. Due to their low purchasing power, the little amount of money spent on food could result in poor nutrition and anemia, especially in the pregnant women.

Table 31
Hematological Data of Orang Asli Pregnant Women at the Gombak Hospital, Kuala Lumpur.

Orang Asl. group	n	Hb (g/dl) mean ± S.D.	% anemic*	PCV mean ± S.D.	MCHC mean ± S.D.
"deep" jungle	37	11.8 ± 1.50	2.7	35.6 ± 4.37	32.3 ± 1.82
"outside" jungle	241	10.8 ± 1.85	29.0	33.5 ± 5.10	32.1 ± 1.92
combined	278	10.9 ± 5.97	25.5	33.8 ± 5.02	32.1 + 2.26

*Hb <10.0 g/dl
Source: Ong, 1973

Several studies on anemia amongst pregnant women at the Maternity Hospital, Kuala Lumpur were recently reported. The first is a study of 96 women who attended the antenatal clinic at the Hospital. The results were analysed according to trimesters, and are summarised in Table 32. The authors pointed out that with the progression of pregnancy, there is a fall in serum ferritin concentration. The prevalence of low hematological values for the different trimesters are also presented in the Table.

Table 32
Hematological Data of 96 Women at Maternity Hospital, Kuala Lumpur

	Hb (g/dl)		MCV (fl)		MCH (pg/l)		MCHC (pg/l)		Sr. ferritin (ug/l)	
	mean	%<12	mean	%<77	mean	%<26	mean	%<32	mean	%<10
First trimester	12.95	0	90.2	0	30.8	0	34.0	0	86.6	0
Second trimester	11.4	52	87.4	0	29.6	8	33.0	10	12.6	24
Third trimester	10.9	59	83.0	15.2	28.0	30.9	33.0	11.9	11.3	26

Source: George, Adeeb and Ahmad (1980)

A smaller series of 19 women in the same Hospital were studied by Jaffar Ali, Khalid Hassan and Hamid Arshat (1981). Mean serum iron and transferrin saturation values are extracted and tabulated in Table 33. No prevalence rates of low values for these parameters were presented. These authors had determined the corresponding cord blood for the same parameter and investigated into the mechanism of transport of iron to the

fetus in pregnancy. Table 33 also gives the hemoglobin levels of 191 pregnant women at the same Hospital reported by these authors for another study (Jaffar Ali, Khalid Hassan and Hamid Arshat, 1982) - that of folate and vitamin B_{12} status discussed in the following paragraph. 18.7% of women in this series were found to have a hemoglobin level of < 10.5 g/dl.

Table 33
Serum Iron Parameters and Hemoglobin of Maternal Blood at Parturition

	Sr. Iron* (µg/dl)	Sr. TIBC* (µg/dl)	Transferrin* Saturation (%)	Hb** (g/dl)
mean	74.0	310.6	26.4	11.4
SD	35.9	108.6	14.5	1.3
range	15.4 - 142.8	107.7 - 571.4	5.0 - 60.0	6.5 - 14.6
n	19	19	19	191

Source: * Jaffar Ali, Khalid Hassan and Hamid Arshat, 1981
** Jaffar Ali, Khalid Hassan and Hamid Arshat, 1982

The third recent study at the Maternity Hospital, Kuala Lumpur was that of the status of folate and vitamin B_{12} of pregnant women admitted at term at the Hospital (Jaffar Ali, Khalid Hassan and Hamid Arshat, 1982). Cord blood was also analysed for the same vitamins. This is one of the few studies on these vitamins in the country. As can be seen from the summarised results in Table 34, 58.5 percent of these women could be considered to have low serum folate levels, and 32.4 percent had lowered RBC folate levels. In contrast, none of the women were found to have a low serum vitamin B_{12} value. It was however pointed out that the number of subjects studied for this parameter was very small. Table 34 also shows that the cord blood levels of these vitamins were significantly higher than the corresponding levels in the maternal blood. The authors suggested the possible involvement of an active process in the transfer of folates and vitamin B_{12} to the fetus. This mechanism would ensure adequate supply of these nutrients to the fetus even in maternal deficiency.

Table 34
Folate and Vitamin B_{12}
of Malaysian Maternal and Cord Blood at Parturition

	maternal blood	cord blood
a. serum folate (ng/ml)		
mean ± Sd	5.0 ± 3.5	19.9 ± 17.4
range	1.0 — 18.5	5.9 — 68.0
% < 5 ng/ml	58.5	not given
n	104	35
b. RBC folate (ng/ml)		
mean ± SD	338.4 ± 227.5	569.6 ± 444.2
range	70.0 — 845.2	91.0 — 1408.9
% < 160 ng/ml	32.4	not given
n	71	7
c. serum vitamin B_{12} (pg/ml)		
mean ± SD	370.4 ± 114.7	496.6 ± 188.7
range	195.0 — 691.0	351.8 — 975.0
% < 100 pg/ml	0	not given
n	14	9

Source: Jaffar Ali, Khalid Hassan and Hamid Arshat (1982)

This group of investigators continued their work in the area and in a recently concluded study (Khalid Hassan, Jaffar Ali, Hamid Arshat and Noor Laily Abu Bakar, 1984), they investigated the status of serum and red blood cell folate and vitamin B_{12} of about 400 specimens of cord blood from the Maternity Hospital. Results of this study, to be published very soon, should provide further information on the status of these vitamins in the infant.

Yet another study at the Maternity Hospital recently concluded by this author and co-workers (Tee *et al,* 1984) has shown that there was a moderately high prevalence of anemia amongst the 309 pregnant women studied, all of whom were from the lower socio-economic strata. As shown in Table 35, based on Hb and PCV values, 30-40% of the women could be considered anemic; approximately half of them presented with unsatisfactory serum iron, transferrin saturation and ferritin values; 60.9% had low serum folate levels; and about 30% may be considered to be of poor protein nutriture. Anemia in the study population was seen to be related mostly to iron and to a lesser extent, folate deficiency. It was also observed that there were statistically significant differences in the hematological, iron, folate and protein status of the women from the three racial groups of the study population. These indices were observed to be poorest amongst the Indian women,

better in the Malays, and generally best in the Chinese. Birth records of 169 of these women revealed that one of the infants had a birth weight of <2.0 kg; incidence of low birth weight, <2.5 kg, was 8.3%. Although there was a trend of deteriorating hematological, iron and protein status of women from the 0, 1-3 and ≥ 4 parity groups, these differences were not statistically significant.

For obvious reasons, the studies reviewed above were all hospital-based. Some were retrospective, making use of existing hospital records. Another point to note is that they were all concentrated in the Kuala Lumpur area. It is hoped that data from hospitals in other areas of the country would be made available. Hemoglobin is frequently determined as a routine test in most ante-natal clinics. What is needed is of course a standardization of the method, regular quality control to ensure satisfactory results, and a collation of all these data. It is also to be noted that except for the three studies cited above, determination of folic acid and vitamin B_{12} has not been carried out. As discussed earlier on in this review, other hemopoietic nutrients besides iron, especially folic acid, could play an important role in the etiology of anemia in the tropics. It is hoped that other studies would deal with this vitamin more thoroughly in the future.

Table 35
Nutritional Anemia Amongst Pregnant Women: Recent Data from the Maternity Hospital

parameters \ racial groups	Chinese	Malays	Indians	Combined
Hemoglobin				
n	104	109	63	276
mean ± SD (g/dl)	11.48 ± 1.80	11.15 ± 1.51	10.51 ± 1.68	11.13 ± 1.70
% < 11 g/dl	30.8	47.7	58.7	43.8
Packed Cell Volume				
n	104	109	63	276
mean ± SD (%)	36.03 ± 4.58	34.75 ± 4.50	33.67 ± 4.42	34.99 ± 4.60
% < 33%	24.0	31.2	47.6	32.2
Serum Iron				
n	117	121	71	309
mean ± SD (ug/dl)	60.18 ± 35.07	48.45 ± 33.53	47.38 ± 38.88	52.65 ± 35.89
% < 50 ug/dl	45.3	60.3	69.0	56.6
Transferrin Saturation				
n	117	121	71	309
mean ± SD (%)	19.45 ± 9.91	15.87 ± 8.44	15.23 ± 8.39	17.08 ± 9.21
% < 15%	38.5	51.2	54.9	47.2
Ferritin				
n	110	103	67	280
mean ± SD (ng/ml)	21.86 ± 25.34	11.09 ± 8.80	12.16 ± 15.32	15.69 ± 19.02
median	13.5	9.0	8.6	11.0
% < 12 ng/ml	40.9	61.2	62.7	53.6
Serum Folate				
n	104	101	66	271
mean ± SD (ng/ml)	4.70 ± 5.06	3.30 ± 3.83	2.47 ± 2.44	3.64 ± 4.19
median	3.15	2.40	1.60	2.40
% < 3 ng/ml	45.2	66.3	77.3	60.9
Serum protein				
n	117	121	71	309
mean ± SD (g/dl)	6.03 ± 0.35	6.21 ± 0.43	6.14 ± 0.36	6.12 ± 0.39
% < 6 g/dl	36.5	23.1	25.4	28.8
Serum albumin				
n	117	121	71	309
mean ± SD (g/dl)	3.24 ± 0.24	3.11 ± 0.32	2.99 ± 0.30	3.13 ± 0.30
% < g/dl	11.1	25.6	46.5	24.9

Source: Tee *et al*, 1984

7.2.3 Anemia Amongst Malaysian Labourers and Industrial Workers

There have been several studies on anemia amongst labourers, particularly of the estate labour force. Although these studies were mostly carried out some time ago, they are of importance and were surely much appreciated at that time. Take for example the study of Battray in 1981, in which 110 Indian and 221 Chinese "coolies" who had just arrived to work on a rubber estate in Johore were studied. Hemoglobin was determined by Tallquist's hemoglobinometer, stool examined for ankylostoma, ascaris and trichuris infestation and the pigmentation of the papillae of the tongue noted. Based on the results of the study, it was recommended that all newly recruited workers be examined for hemoglobin and helminthiasis, and treated if necessary.

In a study of the vital statistics of some estates in Perak before and immediately after the Japanese occupation, Reed (1947) noted that one of the greatest problems at that time was anemia, and that it appeared to be a more serious problem than before the war. It was said that anemia amongst these Indian labourers was a great cause of prolonged sickness and loss of working power and a great contributory cause of mortality. The cause of the anemia was said to be almost entirely nutritional, with both iron deficiency anemia and "tropical macrocytic anemia" occuring frequently.

Another study carried out after the Japanese occupation was a large scale survey by the British Military Administration reported by Bourne (1949), covering several nutritional indicators. Of 1978 rubber estate labourers and dependents examined in Selangor, 47.3% of males, 61.7% of females and 71.8% of children had a hemoglobin value of less than 60% (Tallquist scale). In Penang and Province Wellesley, in the 851 workers examined, 60-70% of the adult males and females had hemoglobin values of less than 60%. All the workers were said to be suffering from a considerable amount of malnutrition. On the other hand, a similar study on 405 industrial workers (including workers in an automobil repair shop, power station employees and employees of a tin mining company) showed that only 4.2% had hemoglobin values below 60%.

Eagland (1952) reported that in routine inspections of an estate labour force of about 2,700, about "4 - 7% showed clinically gross anemia". The condition was said to be "due to a combination of ankylostoma infestation and undernutrition. The report discussed more detailed investigations and treatment methods for the more severe cases

A more comprehensive study was reported by Lamprell and Cheek (1952). The study was carried out between the end of 1950 and early 1952 on a rubber and coconut plantation, consisting of a labour force of 1375 Indians. The plantations, on the coast of Selangor, were said to be virtually free of endemic malaria. From an examination of the hemoglobin levels of 268 labourers and their dependents (by the cyanmethemoglobin method), it was observed that these levels were below the average for Southern Indian labourers in Malaya. Anemia was said to be particularly common amongst women of child-bearing age and the group of women and men who were doing the heaviest work. It was felt that the anemia was essentially dietary in origin; various measures for improvement were implemented in the plantation concerned. A second survey carried out on 100 of these subjects after the implementation of some of these measures showed significant increase in hemoglobin levels.

Other studies of labourers around the time were those of Tasker (1951) and Tasker, Wheland and Cheek (1952). These reports dealt with smaller series of labourers and were mainly concerned with detailed investigations into their anemic conditions and hence will be discussed elsewhere in this review. Burgess and Laidin (1950) in the study of various occupational groups in Malacca also paid attention to a group of labourers.

In a more recent study, Kandiah and Lim (1976) reported on the nutritional status of labourers in a rubber estate community. Of the 78 adult males examined for hemoglobin concentration, 20 (25.6%) were said to be anemic; 72 of the 105 adult women examined (68.6%) were anemic.

In 1978, some data on nutritional biochemistry of industrial workers in Shah Alam, Selangor, were reported (Ng, 1978) (Table 36). This was part of a larger study carried out by the Ministry of Health to obtain baseline information regarding the health and socio-economic status of the workers. Some hematological data were also reported for 135 male and 85 female workers. While only 10% of the male workers had low hemoglobin levels (< 13 g/dl), a much larger prevalence of 26% was observed for the females (< 12 g/dl). Mean values of the serum iron and percent transferrin saturation of the female workers were also found to be lower than that of the male workers. The author expressed concern over the anemia problem faced by these workers, particularly the women.

Table 36. Some Biochemical Parameters of Shah Alam Industrial Workers

	age (years)	serum protein (g/dl)	serum albumin (g/dl)	Hb (g/dl)	PCV (%)	serum iron (µg/dl)	transferrin saturation (%)
Males							
mean	27	7.6	5.1	14.7	45	168	41
SD	7	0.6	0.5	1.4	5	56	12
Females							
mean	24	7.7	4.9	12.7	39	143	32
SD	4	0.6	0.9	1.2	4	43	9

Source: Ng, 1978

8 SEQUELAE OF NUTRITIONAL ANEMIA

Anemia remains a major problem in both developed as well as developing nations. This wide-spread prevalence of anemia as evidenced from the previous section has therefore led to questions of its possible effects on the individual as well as on the community as a whole. The World Health Organization has long recognized that anemia impairs health and working capacity and leads to economic loss, but there is no precise information as to the extent to which it contributes to morbidity in a community (WHO, 1959). Over the last 10 years, several manifestations of anemia that are of clinical as well as economical importance, particularly with reference to iron deficiency, have been recognized. This section will discuss some of the recent studies illustrating some of these manifestations and effects of the anemic condition. Only studies in humans will be cited; no mention will be made of the numerous studies that have been carried out in experimental animals. Most of the studies encountered in the course of this review of the literature have been carried out on iron deficient subjects. This particular emphasis on iron compared to the other hemopoietic nutrients is understandable since most of the nutritional anemia has been known to be due to iron deficiency.

8.1 CLINICAL SYMPTOMS

Various signs and symptoms have often been associated with or ascribed to iron deficiency with or without anemia. These include the well-known epithelial changes of koilonychia (spooning of the nails), atrophy and inflammation at the corners of the mouth (cheilitis), atrophy of the mucosa of the tongue, soreness of the mouth and/or tongue or difficulty in swallowing (dysphagia), pallor, tachycardia and pica. Other symptoms are more vague, but nonetheless real to patients, such as fatigue, delibility, depression and irritability (Heilmeyer and Harwerth, 1970; Fairbanks, Fahey and Beutler, 1971a; Fielding, 1975; Fairbanks, 1978). Studying severe anemia in pregnant women in the Maternity Hospital, Kuala Lumpur, Lourdenadin (1964)

had also reported the observation of these symptoms amongst these women. Various studies of Elwood and associates (described in Elwood, 1970) had repeatedly failed to detect an association between hemoglobin levels and the severity of symptoms or a beneficial effect of iron on symptoms. As summed up by Moore (1970) at the conclusion of the Clinical Symposium on iron deficiency in Switzerland in 1969, "there were conflicting evidences about the specificity of signs and symptoms of iron deficiency and it had not been possible to explain the complaints of patients in adequate physiological or biochemical terms or be sure how causally they are related to iron lack". Our understanding on the subject has not improved greatly since then (Fielding, 1975), and studies cited by Baker and DeMaeyer (1979) were said to have failed to demonstrate clearly that these symptoms were related to iron nutrition.

With regards to megaloblastic anemia due either to folate or vitamin B_{12} deficiency, it has been observed for some time that a characteristic hyperpigmentation of the skin and mucous membranes may develop (Baker, 1966). This hyperpigmentation may be seen all over the body, but has been described by Baker (1981) to be specially marked on the dorsum of the hands and feet, over the terminal phalanges and the interphalangeal joints. Other areas that may also be involved are the nail bed, the palmer aspect of the hands, the soles of the feet and pressure points anywhere in the body. Usually the exposed areas of the body are involved, but Baker (1981) has also described such pigmentation in the oral mucosa and the tongue. The hyperpigmentation is due to excessive deposition of melanin in the basal layers of the skin and mucus membranes and would clear with correction of the vitamin B_{12} or folate deficiency (Hoffbrand, 1978; Baker, 1981). The condition is also said to be more prevalent in subjects who already have pigmented skins such as Indians, Chinese and Africans (Baker, 1981). In his experience with megaloblastic anemia in Africa, Masawe (1981) observed that patients may have premature greying of hair, fever, glossitis and angular stomatitis. Symptoms and signs related to the gastrointestinal tract are said to be common for folate deficiency, particularly sore tongue, angular cheilosis, loss of appetite, or diarrhea (Hoffbrand, 1978). Clinical distrubances of the central nervous system of one form or another was thought to be common in vitamin B_{12} deficiency; this has been reviewed by Baker (1967) and Chanarin (1979). These are believed to be very rare now, since the deficiency state is usually discovered much earlier than in previous times (Hoffbrand, 1978).

8.2 EFFECTS ON PREGNANCY

Though anemia in pregnancy has been a concern to the obstetrician for some years, few data exist to clearly demonstrate that the condition places in increased risk to the fetus and mother. Early studies on severe anemia in pregnancy have shown that there is an associated increased risk of premature

delivery and increased maternal and fetal morbidity and mortality. Most of these have been reported some years back, for example, the studies of Gatenby and Willie (1960) for a series of 100 patients observed from 1953 to 1958 in Dublin; MacGregor (1963); Roszkowski, Wojcicka and Zaleska (1966) of 486 women in a Warsaw hospital; and that of 60 patients in Melbourne by Whiteside, Ungar and Cowling (1968). Other studies were those carried out locally in Maternity Hospital, Kuala Lumpur, namely those of Tasker (1958b), Llewellyn-Jones (1965) and Lourdenadin (1964 and 1969). Results of these will be outlined below to illustrate the possible effect that anemia may have on the fetus as well as on the mother.

8.2.1 Labour and the Puerperium

Various effects of anemia on labour and the puerperium were reported by Lourdenadin (1964 and 1969) upon analysis of records for a series of 510 severely anemic patients (hemoglobin <6.5 g/dl) at the Maternity Hospital, Kuala Lumpur from 1960-1962.

Spontaneous termination of pregnancy before 28 weeks occured in only 21 patients, giving an incidence of 4.1%. It was concluded that anemia did not appear to give rise to an increase in abortion rate. The prevalence of pre-eclamptic toxemia was reported to be 7.5%. This was said to be significantly higher than the overall rate of 4.5% for all cases admitted into the hospital. The breakdown for the 3 years are as follows (Table 37):

Table 37
Pre-Eclamptic Toxemia Amongst Severely Anemia Pregnant Women

year	no. of anemic patients	pre-eclamptic toxemia			
		no of cases			
		mild to moderate	severe	total	prevalence rate (%)
1960	212	3	9	12	5.7
1961	126	5	7	12	9.5
1962	172	9	5	14	8.1
combined	510	15	21	38	7.5

Re-tabulated from: Lourdenadin, 1964 and 1969.

An average of 18 cases (1.6%) of accidental hemorrhage was reported No increase in the incidence of placenta previa was observed; it occurred in only 0.4% of the series of patients studied.

Excluding such other causes of premature labour as pre-eclamptic toxemia, twin pregnancies, fetal abnormalities, ante-partum hemorrhages, there was a high prevalence of premature labour with no other obvious cause except presence of anemia. It was observed that (results tabulated in Table 38) only 16.1% in the women studied went to full term, slightly over half were delivered between 36 and 39 weeks and 26.1% at under 36 weeks.

With regards to labour, it was generally observed to be spontaneous, easy and of short duration. The rate of forceps delivery was 2.9% for these anemic patients, much lower than the 10.0% overall rate for all deliveries. This was thought to be due to the high proportion of premature labour and small sized babies born to these patients. Using the criterion of blood loss of 20 ounces or more, the prevalence of post partum hemorrhage was reported to be low (1.8%). However, the patients were generally more prone to shock, and the need for blood transfusion was greater. Eighty-four of the patients had purperal pyrexia, giving a high prevalence of 16.5%. In spite of this, there were unexpectedly no cases of phlebo-thrombosis in the series of patients analysed.

Table 38
Severe Anemia and the Duration of Pregnancy

year	no. of anemic patients	duration of pregnancy (week)				
		term	36-39	28-36	20-28	<20
1960	212	32	118	47	6	9
1961	126	31	62	20	2	11
1962	172	19	115	26	4	8
total	510	82	295	93	12	28
% of total		16.1	57.8	18.2	2.4	5.5

Retabulated from Lourdenadin, 1964 and 1969.

8.2.2 The Fetus

Tasker (1958b) reported the study of the outcome of pregnancy of 1236 anemic patients and compared with that obtained for over 20,000 non-anemic cases at the Maternity Hospital, Kuala Lumpur. As shown in Table 39, a higher proportion of premature babies (19.6%) was reported for anemic mothers than from those who were not anemic (7.2%). There was also a greater fetal loss (13.2%) from the former group than the latter (6.4%). Overall, a higher percentage (93.6%) of live births was obtained for the non-anemic group as compared with the anemic mothers (74.5%).

Table 39
Severe Anemia and the Outcome of Pregnancy

outcome of pregnancy	infants born of mothers			
	anemic (Hb < 6.5 g/dl)		not anemic (Hb > 6.5 g/dl)	
	number	%	number	%
Fetal loss				
premature	92	7.4	1,001	3.8
mature	71	5.4	675	2.6
total deaths	163	13.2	1,676	6.4
Alive				
premature	151	12.1	907	3.4
mature	922	74.6	23,859	90.2
total live births	1,073	86.8	24,766	93.6
total (27,678)	1,236		26,442	

note: a. fetal loss included still-births and neo-natal deaths
b. prematurity included pre-viable infants with births weight 500 - 2000g
c. the women were grouped based on Hb determination at time of admissions

Source: retabulated from Tasker, 1958b.

Lourdenadin (1964, 1969) examined the outcome of pregnancy of 1063 anemic cases (hemoglobin < 6.5 g/dl) at the same hospital, but for the period 1957-1961, and reported a total "perinatal mortality of 15.5%". Fetal weights tabulated by the investigator (Table 40) shows that weights of the new-borns of these anemic mothers were generally low, with 17.1% below 2 kg and 42% below 2.5 kg. A greater than expected number of twin pregnancies was also reported for the series studied. The incidence was 2.9%. It was found that anemia did not seem to increase the development of congenital abnormalities in 510 subjects with severe anemia.

Llewellyn-Jones (1965) also reported on the extent of fetal mortality and loss in a series of over 70,000 mothers, for the period 1953-1962. The results are re-tabulated in Table 41. It was suggested that the main effects of maternal anemia on the fetus appeared to take place in utero, leading to a disproportionately high stillbirth rate and an increase in premature births.

Table 40
Severe Anemia in Pregnancy and Birth-weights

	number with the following fetal weights (kg)[a]			
year	<2.0	2.0-2.5	2.5 - 3.0	>3.0
1957	37	53	56	60
1958	41	48	60	53
1959	41	90	97	80
1960	27	48	69	68
1961	36	26	36	37
total =1063	182(17.1%)[b]	265(24.9%)	318(29.9%)	298(28.0%)

[a] weights originally reported in pounds have been converted to kilograms;
[b] figures in brackets refer to the percentages of cases in each class of fetal weight to the total number of cases.
Source: re-tabulated from Lourdenadin, 1964 and 1969

Table 41
Severe Anemia and the Outcome of Pregnancy
(Llewellyn-Jones, 1965)

	infants born of	
outcome of pregnancy	anemic mothers[a]	non-anemic mothers
premature rates[b]	18.1 %	5.7 %
perinatal loss	13.1 %	6.8 %
- mature babies	4.5 %	2.3 %
- premature babies	51.6 %	57.0 %
stillbirth rate	91.0 per 1000	15.7 per 1000
neonatal death rate	43.1 per 1000	40.3 per 1000

[a] Hb < 6.5 g/dl
[b] "premature" includes a birthweight between 500g and 1999g.
Source: Llewellyn-Jones, 1965.

8.2.3 Maternal Mortality

Results of the observations of the effects of anemia on maternal mortality for the studies reported by Tasker (1958b), Lourdenadin (1964 and 1969) and Llewellyn-Jones (1965) have been combined and summarised in Table 42. Both Tasker (1958b) and Llewelln-Jones (1965) reported a significantly higher maternal mortality rate for the anemic mothers, compared with the non-anemic group. Hence it appears that anemia not only affects the infant, but also is an added burden to the mothers. It was emphasized that (Llewellyn-Jones, 1965) although in many of these women anemia was not the only cause leading to a fatal outcome, it was a major contributory factor in all of them.

Table 42
Severe Anemia in Pregnancy and Maternal Mortality

	number of anemic [a] mothers			number of non-anemic [a] mothers	
	Tasker (1958) for 1953-1957	Lourdenadin (1964, 1969) for 1957-1961	Llwellyn-Jones (1965) for 1953-1962	Tasker(1958) for 1953-1957	Llwellyn-Jones (1965) for 1953-1962
deaths	21	13	35	111	248
alive	1,205	1,050	2,215	26,157	70,550
total	1,226	1,063	2,250	26,268	70,798
mortality rate (per 10,000)	17.1	12.2	15.6	4.2	3.5

[a] Hb < 6.5 g/dl

Sources: summarised and retabulated from Tasker, 1958; Lourdenadin, 1964 and 1969; and Llewellyn-Jones, 1965.

8.2.4 Specific Effects of Iron, Folate and Vitamin B_{12} on Pregnancy

There has been contradictory reports on the possible effects of specific iron, folate and vitamin B_{12} deficiencies on pregnancy. Some of these studies will be briefly discussed below.

Although it has been long held that iron stores in the fetus will be dependent on the maternal iron stores (Strauss, 1933), reviewing the then available literature, Lanzkowsky (1961) observed that there was a controversy as to whether maternal iron deficiency anemia caused anemia in infants soon after birth. Studying a small series of 59 mothers and their newborn infants in Cape Town, South Africa, the investigator reported that there was no significant difference of mean hemoglobin level in the infants during the first 24 hours of life or at 3 months of age, born to either the anemic or non-anemic mothers. Similar views that maternal iron deficiency had no effect on the hemoglobin level of the baby was also held by Sood *et al* (1975) in a more recent study. In a WHO sponsored collaborative study on nutritional anemia in India, involving over 600 women, these investigators found that supplements of iron, folate, and vitamin B_{12} given to expecting mothers gave a rise in hemoglobin values in these women, but there were no detectable effects on the hemoglobin concentrations in their infants, measured at 3 months of age. Burman (1971) had suggested that the iron content at birth depends a great deal on birth weight, the cord hemoglobin concentration and the degree of feto-placental transfusion. These effects (Baker and DeMayer, 1979) could very well override the effects, if any, of maternal iron deficiency. In another WHO collaborative study of 1000 pregnant women in Southern India reported by Yusufji, Mathan and Baker (1973), it was found that maternal serum iron, folate, and B_{12} concentrations showed no significant correlation with fetal birthweight. It was also noted in this study that supplementation did not give rise to a difference in birthweight. Similar findings were also reported by Shott and Andrews (1972) who showed that hemoglobin concentration of babies born to iron deficient and non-deficent mothers were not distinguishable at birth, and (Sturgeon, 1959; Murry *et al*, 1978) that they remained so during the remainder of the first year of life. Estimates of neonatal iron stores, based on serum ferritin measurements reported by Rios *et al* (1975) also showed little or no difference between groups of infants whose mothers had iron deficiency anemia and those who were not anemic

Exceptions to the above findings are few. In the study of Singla *et al* (1978), 69 sets of anemic mothers, newborn infants and placentas were studied in Varanasi, India. It was reported that the hemoglobin and iron levels in the cord blood and placental tissue had a linear correlation with the maternal hemoglobin levels. Results obtained were thought to suggest that iron supply to the placenta and the fetus was affected in maternal anemia and the fetus took iron in direct proportion to the levels available in the mother.

Rothmans (1970) in her review had discussed several conflicting claims regarding the role that folate deficiency plays in relation to a variety of obstetric conditions such as abruptio placentae, abortion, fetal malformation, still-birth and neonatal death, prematurity and low birth weight, toxemia and post-partum hemorrhage. Isolated claims that vitamin B_{12} deficiency in the infant is associated with B_{12} deficiency in the mother has also been reported (e.g. Jadhav *et al,* 1962)

Several local studies had also investigated the effects of deficiency of these hemopoietic nutrients on the fetus. In the study of Jaffar Ali, Khalid Hassan and Hamid Arshat (1981) of maternal and cord blood iron levels it was found that the fetus takes iron from the mother in amounts proportional to the iron available in the maternal circulation. The amount of iron in the maternal circulation is thus important in determining the iron level in the fetus. On the other hand, it was observed that the availability of iron to the fetus is not directly depedent on the maternal iron stores. In another study, these authors (Jaffar Ali, Khalid Hassan and Hamid Arshat, 1982) observed that the availability of folate to the fetus is also dependent on the folate levels in the maternal circulation. These investigators also reported that there was no correlation between infant birthweight and serum or RBC folate levels at term. No obvious congenital malformations were observed even in the infants born to severely folate deficient mothers. Tee *et al* (1984) also reported no correlation between birthweight of infants and hemoglobin, serum iron and folate levels of their mothers.

It must be emphasized that these findings should not be construed as minimizing the importance of iron, folate and B_{12} nutrition in pregnant and lactating women. However the findings presented seem to show that treating the mother with hemopoietic nutrients may be of more demonstratable benefit to her than to the fetus.

8.3 WORKING EFFICIENCY AND PRODUCTIVITY

Although investigators have long held the view that anemia reduces work efficiency, objective evidences have been difficult to obtain, and scientific documentation of this has been relatively scanty (Baker and DeMayer, 1979; Woodruff, 1982). Several groups of investigators have however in recent studies, provided some evidences that are of interest.

Earlier studies have shown that anemia reduces oxygen carrying capacity of the blood, thereby reducing oxygen delivery to the tissues during exercise, and hence may limit work performance (Sproule, Mitchell and Miller, 1960; Anderson and Barkve, 1970; WHO, 1975). Various later studies attempted to demonstrate that even mild anemia in man could result in decreased performance under a standard applied work load. Ekblom, Goldberg and Gullbring (1972) demonstrated that loss of a relatively small

percentage of total body hemoglobin (by removing blood from the subjects) resulted in a corresponding impairment in performance of a brief but intense exercise task (running on a tread-mill) given to a group of students, and a concomitant decrease in maximal oxygen uptake. After reinfusion, it was observed that there was an increase in both physical performance capacity and maximal oxygen uptake that correlated with the increase in hemoglobin concentration. In a study of agricultural labourers in Central America, Cifuentes and Viteri (1972) showed that in the group receiving oral iron, the Harvard Step Score improved alongside rise in PCV values. Davies, Chukweumeka and Van Haaren (1973), studying a group of industrial workers in Dar es Salaam, Tanzania, working on a bicycle ergometer, observed a marked reduction in maximum aerobic power output and increased cardiac output and plasma volume in anemic subjects, and that (Davies and Van Haaren, 1973) treatment with oral iron could reverse the observed debilitating effects of anemia. These investigators have emphasized that these findings have important implications for developing countries where anemia is endemic, and economic and social development depends largely on subsistence agriculture and physical labour.

Further support for these conclusions came from the studies of Viteri and Torun (1974) who evaluated performace in the Harvard Step Test among Guatemalan sugar-cane cutters, where impairment of performance was observed even with the mildest degree of anemia. Upon treatment with iron, hemoglobin values showed an increase, and there was a parallel increase in performance in the Test. Work performance of a group of female workers in a tea plantation in Sri Lanka (Ceylon) was reported by Gardner *et al* (1977) a few years later. As with previous investigators, the test (with a standard multistaged treadmill) was a near maximal exercise of short duration. Based on four commonly used indicators of work performance capacity: total work time, percent of the subjects who reached the maximal work load, heart rate response to work and post-exercise blood lactate, the investigators concluded that the anemic subjects clearly had a lower work tolerance than subjects with a normal hemoglobin level.

From the studies outlined above, it appears that anemia impairs performance in a brief, intense type of exercise, that the impairment is roughly proportional to the degree of anemia, and that treatment with iron corrects the abnormality. However, since the physical effort required in most occupations seldom approaches near-maximum exercise (WHO, 1975), the economic importance of these observations could not be clearly demonstrated. It is thought that prolonged activity of the endurance type is probably more closely related to job performance and productivity. Daily work productivity would be of particular importance in developing countries where anemia is most common and where nutrition intervention programmes are easier to justify if they are likely to bring about tangible economic benefits (Dallman, 1982).

Recently, studies attempting to evaluate the effect of anemia upon work efficiency in actual industrial settings have been reported. In a study of 78 sugar-cane cutters in Tanzania, Davies (1973) reported a significant association between maximum aerobic power output (measured whilst exercising on a bicycle ergometer) and the daily rate of working in the cane fields (determined from weight of cane cut): high productivity workers had an advantage of maximal oxygen uptake over low producers. These findings were also found to be related to absenteeism although the investigators admitted that this aspect is particularly difficult to quantitate.

Basta *et al* (1979) reported a study of workers on an Indonesian rubber plantation where prevalence of anemia in the population (defined as a hemoglobin value below 13 g/dl) was found to be over 45% and about 88% had hookworm infestations. Productivity of these rubber tappers (mearsured by the amount of wet latex they brought into the weighing hut) were compared for the anemic and non-anemic groups before the intervention, and between the group given oral iron and the placebo group. It was found that before intervention, there was a significantly higher productivity amongst the non-anemic group of workers. After treatment with iron (and a small payment to increase their daily income), it was observed that the anemic tappers raised their productivity significantly and there was a parallel raise in hematologic parameters. There was also an observed significant decrease in the prevalence of infections in this group of workers. These investigators felt that a successful, large-scale programme to reduce anemia in a population such as in Indonesia would have substantial total benefits beyond work output results.

In the same year, Edgerton *et al* (1979) reported a similar study of the effects of iron deficiency anemia on productivity of 199 workers in a tea plantation in Sri Lanka. The quantity of tea picked per day, as a measure of productivity, was studied before and after iron supplementation or placebo treatment. There was a reported significant increase in productivity in association with iron treatment, the effect being particularly pronounced among the more anemic subjects. The effects of the iron treatment on voluntary activity was also estimated in a subgroup of 9 pairs of workers by using a small movement-sensitive recording device that was strapped 24-hours to the subject's body. It was found that subjects who received the iron tablets were significantly more active 2 and 3 weeks after the start of the treatment than their matched pair who received the placebo. From these activity data, the investigators felt that this was the first time that quantitative evidence had been put forth to show that the clinical symptoms commonly attributed to iron deficency anemia, namely tiredness and weakness, may exist in man as had been reported for rats.

Both groups of investigators emphasized the economic implications of increased work productivity with iron treatment, particularly in developing

countries. These studies are thought to provide strong support fot intervention programs to prevent nutritional anemia, such as iron fortification and supplementation in developing countries.

8.4 RESISTANCE TO INFECTION

There has been considerable attention on the subject of the relationship of anemia, especially iron deficiency, to infection. It has been generally considered that anemic individuals are more susceptible to infections. The World Health body (WHO, 1975) felt that there is increasing evidence that anemia and iron deficiency may play a role in limiting the ability of the individual to resist infection. On the other hand, this purported role of iron in host defence is now viewed with skepticism by a new generation of clinicians (Luken, 1975), who claim that available data are often contradictory (Pearson and Robinson, 1976) and that in many instances, the reports are vulnerable to critical review (Strauss, 1978). In this section some studies to illustrate both schools of thought on the problem will be presented. It will be noted that most of these studies were on infants and young children and the infectious diseases looked for were frequently acute respiratory and gastrointestinal infections, both of which are known to occur particularly in this vulnerable group. As pointed out by Baker and DeMayer (1979), there have been few reports on the relationship between anemia and infection and none based on good community studies.

8.4.1 Infection Frequency

One of the frequently quoted and earliest studies on the effects of iron on rates of infection was that of Mackay (1928). In this study, where infants of the lower socio-economic group were examined, she reported that iron-supplemented infants experienced fewer episodes of bronchitis and gastroenteritis than those who did not receive iron. Further, it was her impression that the rate of recovery was considerably better in the iron group than in the control group. Luken (1975), and Pearson and Robinson (1976) have however pointed out defects in the design of the study, such that alternative interpretations of the observed difference could be made. It was specifically pointed out that in using retrospective control data, variations in annual differences of prevalence of infectious disease was not taken into account and that there was no statistical evaluation of the results.

In a more recent report, Andelman and Sered (1966) conducted a prospective study amongst 1048 infants from the lower income group in Chicago. Randomly selected infants were fed either a cow's milk formula fortified with iron and vitamins or one supplemented only with vitamins. The two groups of infants were followed for 18 months and data on growth, iron metabolism and rate of respiratory infections were recorded. It was reported

that from 9 to 12 weeks of age onwards, the iron supplemented group had a significantly higher hemoglobin concentration than the control group. Furthermore, the incidence of respiratory infections in the former group, while receiving iron was significantly lower (about half) than that in the control group, and that this lower incidence extended for several months beyond discontinuance of the iron-containing milk formula. This frequently cited study has been similarly criticized by Luken (1975), and Pearson and Robinson (1976). In their reviews, these authors pointed out that the investigators of the study did not define the criteria used for the diagnosis of infection and that since the collection of data depended on the recall of illnesses by parents over a long interval, their reliability might be questioned.

In a retrospective study, Shaw and Robertson (1963) were able to provide some indirect evidence to suggest iron deficiency as a cause of increased susceptibility to infection. It was reported that two-thirds of the hospitalized anemic children (between 6 months and 24 months of age) had evidence of infection. Lovric (1970) reported that iron deficiency anemia (defined as a hemoglobin level of 10 g/dl or below, in association with a microcytic, hypochromic blood film) was found in only 3%of children (6 — 36 months) attending baby health centres in Sydney. This was said to be in contrast to a group of hospitalized children of the same age group where the rate was found to be 20% This finding was thought to provide circumstancial evidence linking iron deficiency anemia and childhood morbidity. These two studies can of course at best only provide suggestive evidence, and as pointed out by Pearson and Robinson (1976), the cause and effect relationship is not at all clear.

On the other side of the story, several studies have been reported. One such earlier report is that of James and Combes (1960), where it was found that 84 premature infants who were given an intramuscular injection of iron had higher concentration of hemoglobin, but failed to show any reduction in infection rate as compared with 97 controls not given iron. However, Baker and DeMayer (1979) had pointed out that premature infants are notoriously prone to develop infections and this may have masked any deleterious effects of anemia. It was also noted that the number of infants studied was too small so that only relatively large differences in morbidity could have been detected. On the other hand, the study of Salmi, Hanninen and Peltonen (1963) on a group of 95 Finnish premature infants seem to provide contrasting results. It was reported that iron-supplemented infants not only had a higher hemoglobin level, but also had a two-fold reduction in the number of infections at 6 months of age when compared to infants who did not receive iron.

An often quoted study to demonstrate the lack of any effect of anemia on the incidence of infection is that of Burman (1972), where 450 English infants "above average in maternal care and social stability" were divided into

two study groups. One was given iron supplementation and the other was not. Observing these children for 3-24 months, it was found that there was no relationship between the rate of infection and iron supplementation. Again, Baker and DeMayer (1979) had criticized the study, pointing out that the results would not be unexpected since the difference in hemoglobin between the two groups was only 0.09 g/dl. At the same time, Pearson and Robinson (1976) felt that the study suffered from the same methodologic defects as that of Andelman and Sered (1966) (discussed above) in that data collected were based on parent recall and criteria for infections were not defined.

Another study that is perhaps worth mentioning is that of Masawe, Muindi and Swai (1974). It is one of the few studies on the subject carried out on adults. The 110 African patients with hemoglobin< 10 g/dl studied for frequency of infections were separated into two groups: one with severe iron deficiency anemia and dimorphic anemia and the other group with other types of anemia (including megaloblastic, hemolytic and refractory). It was observed that anemia was indeed associated with a high frequency of infections. But it was noted that anemic patients with negative iron stores had significantly less bacterial infections than those in the second group, i.e. those with positive iron stores. Conversely, patients in group 2 had significantly less parasitic infections (e.g. malaria) than patients in the first group. Baker and DeMayer (1979) however, viewed these findings differently and suggested that this study cannot be taken as suggesting either that iron deficiency confers any protection against infection, or that iron administration per se increases the incidence of malaria.

8.4.2 Immunological Status

8.4.2.1 Phagocytic Function

Two components of the normal phagocytic process are recognized: engulfment, which depends mainly on glycolytic metabolism, and intracellular killing, which depends on the hexose monophosphate pathway and oxidative metabolism. In the latter process, it is thought that myeloperoxidase (MPO) an iron-containing enzyme, together with a peroxide system constitutes an important intracellular bactericidal mechnism in these phagocytes (Sbarra *et al,* 1974). Seth and Chandra (1972) and Sbarra *et al* (1974) had demonstrated that phagocytic engulfment and/or killing processes are depressed in severe malnutrition, although it was not possible to ascribe the effects to specific essential nutrient (s).

Higashi *et al* (1967) had oberved a decreased mean cellular peroxidase in phagocytic cells of iron-deficient individuals. Arbeter *et al* (1971) studying a group of patients with various degrees of malnutrition, including iron deficiency, reported that iron utilization and iron deficiency affected adversely intracellular bactericidal killing and suggested that the iron deficiency state

may affect the MPO essential for intracellular killing of bacteria. The clinical relevance of impaired MPO activities are however unclear (Pearson and Robinson, 1976). Another study using the *in-vitro* method of measuring bactericidal capacity of leucocytes was reported by Srikantia *et al* (1976). These investigators reported a significant depression of this defence capacity in a group of anemic (said to be mostly of the iron-deficient type) children with hemoglobin < 10 g/dl. The impaired phagocytic bactericidal capacity observed by Chandra (1973) and Chandra and Saraya (1975), which was in general correlated with the severity of the iron deficiency state (in the latter report), was said to have been reverted to normal within 4-7 days after treatment with iron, before any appreciable change in hemoglobin concentration had occurred. Similar decreased bactericidal capacity of iron-deficiency anemia children was also reported by Macdougall (1975).

On the other hand, several other research groups have found no abnormality in phagocytic activity in patients with iron deficiency. Eight Thai children (aged 3-4 years) were studied by Kulapongs *et al* (1976), all with severe iron deficiency (and hookworm infestation) but no evidences of folic acid and vitamin B_{12} deficiencies. It was found that only one of the 8 patients had a depressed phagocytic killing function. A few years earlier, Masawe and Nsanzumuhire (1973) had also reported no decline in anti-bacterial activity of serum from 12 African patients with iron-deficiency anemia (hemoglobin < 8 g/dl). There was, however, a sharp decline of this capacity in a group of 8 patients with sickle cell anemia.

Pearson and Robinson (1976) commented in their review that the results of the various reported studies are difficult to compare. Investigators had used different target organisms in their assay of bactericidal activity such as *Staphylococcus aureus, E. coli* and *S. albus*. Furthermore, the inoculum size used in the various test systems either varied or was not described.

8.4.2.2 Cell-Mediated Immunity (CMI)

Host resistance to intracellular organisms, whether bacteria, fungi, protozoa or viruses, is thought to be mediated principally by specifically sensitized thymus-dependent lymphocytes (T cells) (Pearson and Robinson, 1976). Investigators have used various methods to evaluate this cell-mediated immunity (CMI). The classical means is by the *in vivo* method of skin reactivity (delayed cutaneous hypersensitivity) to antigenic challenge. *In vitro*, lymphocyte responsiveness to mitogens such as phytohemagglutinin (PHA) may be monitored. Recently, evaluation of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis, for example by determining the incorporation of tritiated thymidine (^{3}H-thymidine) into DNA has also been used. CMI function has been shown to be depressed in protein calorie malnutrition

children and adults in various studies (e.g. Smythe *et al,* 1971; Chandra, 1972; Edelman *et al,* 1973; Law. Dudrick and Abdou, 1973; Schlesinger and Stekel, 1974). The effect of specific nutrient deficiency, including iron, folate and vitamin B_{12} have not been well defined. Some studies attempting to examine the possible effects of these nutrients, especially iron, are outlined below.

One of the earliest studies in this area was reported by Joynson *et al* (1972). In 12 adults with iron deficiency, the investigators observed a poor cutaneous hypersensitivity to Candida antigen and to purified protein derivative (PPD). *In vitro* studies showed that lymphocyte transformation, as indicated by thymidine uptake for DNA synthesis. was significantly impaired in the iron-deficient subjects when PPD was used as the antigen. Production of migration inhibition factor (MIF) was also poorer on this group of subjects. In a later report, this research group (Jacobs and Joynson, 1974) documented that treatment with iron restored skin reactivity and lymphocyte MIF production to normal.

Similar results were reported by Chandra and Saraya (1975) and Macdougall *et al* (1975). Both groups, studying iron-deficient children, reported depressed cutaneous hypersensitivity as well as an impaired stimulation of DNA synthesis in lymphocytes by PHA. The former group of investigators also observed a reduced proportion of T cells in peripheral blood lymphocytes of the subjects. In both studies, the former in India and the latter in South Africa, it was found that treatment with iron restored most of the cellular immune functions to normal. In a somewhat different setting, Fletcher *et al* (1975) reported in the 29 adults with iron deficiency studied (serum levels of folate and vitamin B_{12} were said to be normal), the PHA-induced lymphocyte transformation was impaired and returned to normal after iron therapy. More evidence has been provided by Srikantia *et al* (1976) studying T-lymphocyte count and tritiated-thymidine incorporation in a group of Indian children with iron deficiency anemia.

Commenting on these reports, Pearson and Robinson (1976) pointed out that although most of the subjects in these studies had well-defined iron deficiency states, other mild nutritional deficiencies were not definitely exluded, except in the study of Fletcher *et al* (1975). It is clear that especially in poorly nourished populations, iron deficiency may be associated with, for example folic acid deficiency. Furthermore, evidences have been put forth to suggest that folic acid and not iron deficiency brings about impaired CMI functions. The study of Kulapongs *et al* (1974) appears to point towards this direction. It was observed that in the 8 Thai children who had severe iron-deficiency anemia, but no evidences of folic acid or vitamin B_{12} deficiencies, there was no significant impairment of blast cell formation and *in vitro* incorporation of ^{3}H thymidine into PHA-stimulated lymphocytes. Coovadia *et al* (1974) observed that the depressed PHA-stimulated lymphocyte transformation in

South African children with protein-calorie malnutrition was associated with folic acid deficiency (and responded to treatment with the vitamin) but not with iron lack. A year later, workers from the same institution in South Africa put forth more direct evidences. Gross *et al* (1975) reported that iron deficiency alone did not affect skin reactivity and that lymphocyte transformation was only slighty but not significantly depressed in their study of 23 Africa patients. On the other hand, it was observed that folic acid deficiency alone or in combination with iron deficiency resulted in depressed CMI as determined by skin responsiveness to dinitrochlorobenzene and PHA-stimulated lymphocyte transformation. This finding was further substantiated when it was found that treatment with folic acid restored CMI to normal. The investigators suggested it could be that iron deficiency adversely affected CMI through interference with folate metabolism.

Other studies of the effects of folate and vitamin B_{12} on CMI function are scarce. An example of a study of vitamin B_{12} deficient subjects is provided by that of Das and Hoffbrand (1970). Examining these subjects for PHA-stimulated transformation of lymphocytes, it was observed that there was no difference in the percentage of cells undergoing transformation. However, it was found that the transformed cells had an abnormal morphology described as "megaloblastoid". On the other hand, in the study of Bhaskaram and Reddy (1975), whilst the iron-deficiency anemia children studied had demonstrated depressed CMI functions which improved somewhat with iron therapy, in another group of children with "vitamin B complex deficiency", these functions were not altered. It was thus suggested that vitamin deficiencies may not have an appreciable effect on the immunological status of children.

Hence, it would seem that the effects of iron, folate and vitamin B_{12} on CMI function are not well-defined and no firm conclusions can be drawn. Buckley (1975), Pearson and Robinson (1976) and Strauss (1978) pointed out some of the problems encountered in attempting to compare the results from these various studies: (a) the population groups studied varied widely with respect to age and geography; (b) intercurrent infections, especially of viral etiology, were not always excluded, bearing in mind that some of these are known to cause depression in CMI; (c) differences in laboratory methods used, and inter-laboratory and intra-laboratory variations in some of these methods; and (d) wide variations of lymphocytes from a single subject to a particular stimulant. It was felt that some of these factors could account for the observed discrepancies between the results of the different research groups.

8.4.3 Anemia and Infection: Summary of Current Status

The reviews of Luken (1975), Pearson and Robinson (1976), and Baker and DeMaeyer (1979) on the effects of nutritional anemia (especially with respect to iron) on infection have a similar conclusion: the problem remains unsettled. Although there may be suggestive evidence that anemia in infants make them more prone to respiratory infections (Baker and DeMaeyer, 1979), all three groups of authors had emphasized the need for further studies in this important area. Pearson and Robinson (1976) noted the difficulty of assessing the role of a single nutrient such as iron in such complex syndromes as systemic infection and malnutrition where multiple factors may be operative. Luken (1975) pointed out the dilemma faced by investigators: continued reliance on *in vitro* systems to further characterize the relationship between the nutrient and microbial growth is not likely to provide information relevant to clinical medicine; clinical surveys may not provide us with conclusive insights into the effects of anemia on immune response since the baseline for frequency of infections is high in the age group under study; in retrospective studies, it is virtually impossible to assume that the nutrient under study is the sole variable between the groups of subjects: in prospective studies, ethical considerations necessitate the exclusion of children with even a mild deficiency; and epidemiological studies are no doubt difficult to control and results could be difficult to interpret. However, Elwood (1970) emphasized that there should be no substitute for population samples, and only a global concept of iron deficiency, such as that obtained by an epidemiologist is likely to be unbiased. Weinberg (1978), in a recent review, had emphhasized the need for well-designed epidemiological studies to ascertain whether mildly and severely iron deficient persons are more or less or equally susceptible to infection as compared with iron sufficient (and iron overloaded) individuals. Such studies, it was felt, should include not only hematologists and nutritionists, but microbiologists as well.

8.5 PSYCHOSOCIAL AND DEVELOPMENTAL CONSEQUENCES

Catecholamines have been thought to play a role in influencing human behavior. Any increase in catecholamines was thus thought to contribute in determining behavioral symptoms such as manifested by iron deficient patients (Wagner *et al,* 1979). Vorhess *et al* (1975) had observed increased urinary excretion of norepinephrine in iron deficient children and Wagner *et al* (1979) reported plasma levels of epinephrine and norepinephrine in iron deficient adults. It was largely such observations that stimulated recent interest in the psychological and developmental consequences of iron deficiency (Dallman, 1982).

Several iron-deficent infants and young children have often been characterized as irritable, inattentive and uninterested in their environment.

Some of the studies attempting to verify such clinical impressions have been recently reviewed by Dallman (1982) (some of the studies cited were still in print at the time of the review). These studies were mostly on infants and young children between the ages of 9 months and 24 months, with iron deficiency anemia. They were usually randomly assigned into 2 groups, the iron treated and the placebo groups. Various psychological and development tests were administered before treatment and a second time about a week after the start of treatment, and the results compared. The Baily Scale of Infant Development Tests, said to be widely accepted tests for young children, were commonly used. These tests included items on sensory development, fine and gross motor skills and language development. Details of such a study are given in Oski and Honig (1978).

In spite of the complexities of experimental design and some difficulties in the interpretation of data, Dallman (1982) felt that the studies reviewed were all indicative of some behavioral changes in association with iron deficiency. However, there appeared to be some disagreement about the rapid reversibility of the abnormalities. Dallman (1982) also pointed out some possible confounding variables of such studies, which are not easy to exclude, such as the presence of other nutritional deficiencies, and poor environmental and social circumstances.

The review Pollitt and Leibel (1979) has discussed some studies of the interllectual function of iron-deficient children, demonstrating varying adverse effects of anemia on one or more cognitive processes. Webb and associates have also carried out some studies into the effects of anemia on scholarstic achievement of individuals. In the study of Webb and Oski (1973), it was obseved that a group of adolescent boys and girls with iron deficiency showed significantly lower scores of scholarstic achievement than their matched but otherwise normal controls. The investigators however noted that from the data it was unclear whether the poor performance observed for the anemic students was a consequence of anemia *per se*, iron deficiency alone, or a result of a general nutritional inadequacy, of which iron deficiency was only one easily identifiable component. Fieldings (1975) pointed out that this type of investigations has the pitfalls of possible multiple confounding variables.

The review of Burman (1982) did not bring out other studies to indicate the role of iron deficiency in psychosocial development. The review of Leibel, Greenfield and Pollitt (1979) was cited, which pointed out that "there exists no unequivocal demonstration of an adverse effect of iron deficiency on in-

telligence, learning, attention, motivation or general sense of well-being". Commenting on the observed associations between scholarstic performance and iron deficiency, Fairbanks (1978) pointed out that it may be that other socio-economic fantors are the root cause of the poor diet, poor growth and poor school performance.

8.6 OTHER EFFECTS

The effect of anemia on growth is another aspect of the problem that has been little studied. In a study of 88 children (less than 3 years of age) with iron deficiency anemia in Philadelphia (with hemoglobin <9g/dl), Judisch *et al* (1966) observed a preponderance of children in the lower weight ranges. These investigators pointed out that non-deficient infants and young children, especially those on a largely milk diet and inadequate amounts of iron-containing solids are often underweight, contrary to popular belief that these children are often "fat, pale and flabby". They were able to demonstrate that with iron therapy, this type of underweight could be reversed. However, as pointed out by these investigators, it was not posssible to determine whether the reduction in weight observed was a direct result of the iron deficiency anemia, or whether is was secondary to a reduction in food intake due to anorexia. Beard, Finch and Mackler (1981) felt that maternal iron depletion in man has not had any obvious effect on growth and development of the newborn infant. Burman (1982) has suggested that there may be no direct relationship between weight and iron deficiency: iron deficiency and suboptimal weight may be due to a common nutritional problem such as food refusal, food fads or any cause of malnutrition. The mechanism of this relationship, if any, thus remains obscure.

Other structural and biochemical abnormalities associated with iron, folate and vitamin B_{12} deficiencies have been reviewed in Fairbanks (1978) and Baker and DeMaeyer (1979).

9 INTERVENTION MEASURES

In view of the close association observed between high prevalence of anemia and poverty, it may be thought that intervention to improve the standard of living would be accompanied by better diet and hence the eradication of anemia. Fairbanks (1978) has however pointed out that the alleviation of the anemia problem "should not await a hypothetical global rise in affluence". It was further emphasized that because of religious convictions or dietary habits prevalent in some countries, a rise in living standard will not necessarily be accompanied by "better" foods being consumed. Hence, governments, especially of countries with high prevalence of anemias, and the World Health Organization had implemented one or more other forms of intervention measures. The various measures available for the control of nutritional anemia have been grouped into 3 main categories (Baker, 1978; Baker and DeMaeyer, 1979):-

1. therapeutic supplementation with the hemopoietic nutrient or nutrients which are found to be deficient;
2. fortification of the diet with the nutrient(s) which are deficient;
3. ancillarly measures, such as:
   a). reducing nutrient losses, e.g. by hookworm eradication;
   b). increase nutrient absorption through promoters; and
   c). education campaigns.

As a public health measure to deal with the problem of nutritional anemia in the community, these programs would be very much different from the relatively simple case of treatment of anemia of individuals in the clinics or hospitals (Baker, 1981). Due to the failure to appreciate that these measures are not simple matters of supplying the missing nutrient(s), much effort, time and money have often been wasted (Baker and DeMaeyer, 1979). These intervention programs are highly complex and calls for careful planning and execution in a methodical manner that enables its efficacy to be tested step by step (Baker, 1978, 1981). Baker (1978) has emphasized the need for an initial pilot scheme to be carried out, which, if is shown to achieve the desired results, should then be followed by a trial under realistic field conditions. Only when a field trial has proved successful should a national or regional program be introduced. Dallman, Siimes and Stekel (1980) have discussed programs specifically for the prevention of iron deficiency anemia in infancy and childhood, which would necessarily require a rather different approach than those discussed in this section for the community at large.

9.1 THERAPEUTIC SUPPLEMENTATION

Supplementation is the term applied when an extra amount of nutrient is given in medicinal form, whether orally as a tablet or mixture or parenterally by injection (Baker and DeMaeyer, 1979). When there is a high prevalence of anemia, especially due to iron deficiency, the only way to improve the situation within a short space of time (such as during pregnancy) is to provide therapeutic supplements (WHO, 1975; Charlton and Bothwell, 1982). The term prophylactic supplementation may sometimes be encountered. This form of supplementation may be suggested when the prevalence and severity of anemia is lower, and there is more time available for correction of the deficiency states. When food fortification (which is the method usually used for this purpose) is not posssible or practicable, prophylactic supplementation of the diet may be carried out with the necessary nutrient(s), but in smaller amounts than required as therapy for anemic populations. Such supplementation may be given, for example, to premature infants who begin extrauterine life with low iron stores (WHO, 1975). However, the distinction between these two forms of supplementation is not always clear-cut (Baker and DeMaeyer, 1979)

Numerous factors have to be considered in planning therapeutic supplementation for a community. It is important that the supplement be stable under conditions of storage likely to be encountered in the field (Baker and DeMaeyer, 1979), especially with folate tablets (Baker, 1978). It is essential to ensure that the preparation which is chosen is fully bioavailable (Charlton and Bothwell, 1982), bearing in mind that the physical conditions under which the tablet (e.g. iron) are made, the nature of the excipient and the type of coating may all influence the availability of the substance *in vivo* (Baker, 1978). The appropriate dose of supplement should be established, since absorption is dependent on a number of factors and a dose which is adequate for individuals living in one area may be inadequate for those living in another (Baker and DeMaeyer, 1979). Baker (1978) has discussed in some detail the approach to calculating the amount of iron that must be absorbed each day in order to achieve the desired effects within a given period of time. On the other hand, with folate, Baker (1978) pointed out that since the amounts of the vitamin required are not known with the same precision as those of iron, and since the bioavailability of dietary folate cannot easily be measured, the amount of supplement required in a given situation is not so easily calculated. However, it was further pointed out that it may not be necessary to do so with great precision since unlike iron, folate preparations would be readily absorbed, and because tablet production and distribution would cost far more than the folate in the tablets.

Obviously, if oral supplementation is chosen, there is the pre-requisite of identifying the machinery for the distribution of the medication. No specific or so-called "vertical" control programs should be initiated: they should preferably be integrated with other nutrition programs (De Maeyer, 1981), thus saving costs and increasing its efficacy (Charlton and Bothwell, 1982). Nevertheless, as further pointed out by Charlton and Bothwell (1982), even under optimal conditions, it is hardly possible to reach all the affected individuals or to achieve complete compliance, so that the oral supplementation approach can at best only achieve partial success.

Parenteral administration of a supplement has the advantage in that it can overcome the problem of irregular consumption, difficulties of absorption and possible side effects which may be associated with oral supplements. On the other hand, there are also disadvantages associated with parenteral supplementation: the cost of a large-scale supplementation program very high, and parenteral iron therapy has been reported to be associated with severe or even fatal reactions. It was thus felt that from a routine public health point of view, parenteral supplementation would probably not be practicable or justified (Baker and DeMaeyer, 1979).

Example of various supplementation programs (some of which were WHO sponsored) have been reviewed by Baker and DeMaeyer (1979). Implementation of such programs in Mauritius, Israel, Burma and India and

other areas were said to have shown that therapeutic supplementations can effectively increase the hemoglobin concentration in deficient subjects. Much lessons were also said to have been gained from such programs, such as the amounts of supplements that must be given to bring about the desired improvements. DeMaeyer (1981) has also discussed in detail some considerations in the supplementation of iron and folate to pregnant women and young children in developing countries.

9.2 FORTIFICATION

A joint FAO/WHO Expert Committee recommended that the term "fortification" would be the most appropriate to describe "the process whereby nutrients are added to foods to maintain or improve the quality of the diet of a group, a community or a population" (WHO, 1971). This intervention measure offers several obvious advantages (Baker, 1978; Baker and DeMaeyer, 1979):

(a) theoretically, it can be used for increasing the intake of any of the hemopoietic nutrients but most studies have been carried out on iron fortification;
(b) it is applicable to large population groups at a relatively low cost;
(c) since usual channels of food distribution are used, it does not require a special mechanism for delivering it to the intended recipients; and
(d) supervision of its consumption is not essential.

It is clear however, that since the amounts of iron that can be made available to the community by this means is limited, it may be used only as a long term intervention measure in situations where there is a mild to moderate degree of deficiency. Furthermore, it is not so simple as it may seem to be and numerous factors have to be considered.

An appropriate vehicle (i.e. the foodstuff to which the nutrient supplement is to be added) should be selected. The ideal vehicle should be (WHO, 1975; Baker and DeMaeyer, 1969):

(a) one that is already consumed in adequate amounts by the section of the community in need of the fortification;
(b) one that is suitable for fortification on a large scale;
(c) one that is manufactured at relatively few centres so that quality can be adequately controlled and monitored;
(d) one that results in a product with is stable under the extreme conditions of storage and distribution; and
(e) one such that the palatibility of the vehicle or other foods that the vehicle may be mixed with (e.g. during cooking) is unchanged.

Possible vehicles in use, or being explored for use, in iron fortification programs are wheat flour: (in e.g. Sweden, the United Kingdom, the United States and Norway), sugar (in Guatemala and Mexico), salt (in India, In-

donesia, the Philippines, and Thailand), fish sauce (Thailand), sodium monoglutamate (Philippines), curry powder (South Africa), and processed infant foods (Baker, 1978; Viteri *et al,* 1981).

The choice of a suitable compound of the nutrient to be added to the vehicle is of considerable importance. Charlton and Bothwell (1982) have particularly emphasized this and pointed out that the preparation selected in some of the earlier iron fortification programs have proved to be poorly bioavailable. The compound chosen should (WHO, 1975; Baker and DeMaeyer, 1979):

(a) be one that is readily assimilated when mixed with the vehicle, and when the vehicle containing it is added to the diet;
(b) not cause undersirable changes such as colour, odour or flavour to the vehicle and the diet; and
(c) be stable under conditions in which it will be used or stored.

The various forms of iron available for fortification and the factors to be considered in choosing the appropriate form have been discussed in some detail in WHO (1975). Various other aspects of the fortification program, especially iron fortification have been discussed comprehensively in various recent publications (e.g. WHO, 1975; Baker, 1978; Fairbanks, 1978; Baker and DeMaeyer, 1979; Hallberg, 1982; Charlton and Bothwell, 1982).

The fortification of foods with folic acid has not been thoroughly studied. WHO (1975) has suggested that the same considerations for iron fortification should also apply to fortification with folate. However, particular attention should be paid to the effect of food processing, cooking and storage on the folate content. Studies on the fortification of maize, rice and bread have been recently reported by Colman and co-workers (Colman *et al,* 1974, 1975; Colman, Green and Metz, 1975; Margo *et al,* 1975). From studies in South African, these investigators felt that folate fortification is a technical possibility and may be expected to make a significant contribution to the health of pregnant women.

Some investigators have pointed out the need for considering the safety of fortification programs, especially iron fortification (Charlton and Bothwell, 1982), refering to the risk of iron overload in some people. Admittedly, this must be taken into account in the design of any iron fortification program and iron stores should be monitored (e.g. by ferritin assay) to watch for any development of adverse effects especially amongst those who have iron stores in the upper percentiles (Baker and DeMaeyer, 1979). However, Halberg (1982) has emphasized that it should be borne in mind that the amounts of iron used in iron fortification will lead to a dietary intake of iron within a "physiologic" range and that the purpose of the fortification is not to treat already existing iron deficiency anemia, but rather to

prevent the development of the deficiency by a "normalization" of the diet. Futhermore, Baker and DeMaeyer (1979) have pointed out that in most communities, fortunately enough, subjects with iron overload form only a tiny segment of the population and, from the public health point of view, it would be much easier to deal with these individuals than it is to tackle the large number of people with iron deficiency.

On the efficacy of such iron fortification programs, investigators have slightly differing views. Where evaluation studies of iron fortification of wheat flour have been carried out (of which there has been suprisingly few, considering that the fortification has been carried out in a number of countries for some years now), they suggest that some current practices are ineffective, either because the supplement is not adequately absorbed, or the level of fortification is inadequate, or a combination of both factors (Baker and DeMaeyer, 1979). However, these authors pointed out that at least in some situations, there is good evidence that iron fortification of wheat can indeed improve the iron nutrition of the community. Hallberg (1982), citing the example of the Swedish experience, where there has been a marked drop in the prevalence of iron deficiency in women during the last 10 to 15 years, from about 25-30% in the 1960s to about 5-10% pointed out that an analysis of the relative role of various factors bringing about this reduction has revealed that iron fortification of flour has brought about a third of the observed reduction. Fleming (1982) has also exppressed support of the fortification approach and predicted that it is probable that the global control of nutritional iron deficiency anemia could be achieved in the forseable future. Masawe (1981) however is more cautions and has suggested that further evaluations are needed before definite conclusions may be made.

In Malaysia, to date no massive intervention measures, either therapeutic supplementation or fortification of foods with hemopoietic nutrients have been attempted or planned. Ancillary measures in the form of deworming campaigns have been carried out on an ad hoc basis only.

9.3 ANCILLARY MEASURES

As discussed earlier, heavy infestations with helminthic parasites, especially hookworm, could result in excessive iron losses. Measures that can be taken to assist the reduction of nutrient loss would include: (a) attempts to reduce exposures, such as encouraging the use of pit latrines, use of foot-wares, and discourage the use of human feces as fertilizers (Fleming, 1982); (b) decrease worm load by periodical deworming (Baker and DeMaeyer, 1979; Beaton, 1974).

The absorption of non-heme iron is known to be poor and may be influenced by various factors. It would thus be posssible, at least theoretically, to increase absorption of iron through various changes to the diet (Beaton,

1974). However, even though it is known that increased intake of meat, fish and foods rich in ascorbic acid could promote iron absorption, persuading people to change the habits they have adopted, either due to economic deprivation or religious or cultural beliefs, would hardly be a wise approach to the problem (Baker and DeMaeyer, 1979).

9.4 INTEGRATED APPROACH

As pointed out by Baker (1978), the above-mentioned intervention measures are not mutually exclusive, and to achieve succes, it would probably be necessary to combine a number of different measures in an intergrated approach to the problem. Hence, whilst supplementation programs are implemented for groups most at risk, simultaneous fortification programs aimed at the whole or specific sections of the community could be carried out. Control of parasitic infection may be one of the necessary measures. Education of the community, as well as doctors, nurses and other health workers, and politicians should be a continuous and on-going programs. This is a slow and arduous process requiring much time and dedication (DeMaeyer, 1981). It is very often a neglected aspect of public health programs. Viteri *et al* (1981) have emphasized that these programs, like any other health and nutrition programs, should be dynamic in their adoption, in that continuous evaluation and readjustment of the program are necessary.

10 SUMMARY AND CONCLUSION

Anemia is a nutritional problem of immense magnitude, afflicting large population groups all over the world, especially in developing countries. The main hemopoietic nutrients are iron, folate and vitamin B_{12}. The importance of iron to mankind is said to have been recognized even long before medical practice became firmly established, whilst the B vitamins, folate and B_{12} have a more recent history. Iron deficiency is now known to be the commonest cause of anemia, followed by folate deficiency. Vitamin B_{12} deficiency is thought to be a less important cause.

The healthy, adequately nourished individual is normally in a state of nutritional balance. This balance may be disturbed by one or more factors which may lead to a relative or absolute deficiency of the nutrient. As with other nutrients, the balance of these hemopoietic nutrients is a function of a variety of factors, including the amount stored in the body, the rate of loss, the requirement for these nutrients, the amount taken in and absorbed by the body, as well as the rate of utilization. Each of these factors has been well studied. For instance, the storage sites and forms of the three nutrients have been largely elucidated. The major routes of the basal physiological losses of these nutrients have been estimated, thereby enabling the calculation of the body requirements. Since various factors are known to influence the

absorption of nutrients from the diet, there has been some studies into the efficiency of absorption of the hemopoietic nutrients. Dietary requirements have then been estimated.

The basic etiology of a deficiency of the hemopoietic nutrients is a reduction of the absorption of these nutrients, an increased body requirements, or an increased rate of loss from the body, or a combination of these factors. A reduced absorption of the nutrient can occur as a result of a low level of the nutrient in the diet, or because the nutrient has a poor biological availability. At certain periods of life, such as during the rapid growth of the young child or during pregnancy, there is an increase in the requirement for these hemopoietic nutrients. Increased rates of loss of the nutrients, particularly iron, are known to occur with parasitic infections. Thus, for the young child and the pregnant women, especially those in the lower socio-economic segment of the population, hemopoietic balance would be particularly precarious. They would be most vulnerable to hemopoietic nutrient imbalance and the resulting anemia state. Studies in Malaysia have also shown that these are the major etiologic factors giving rise to the nutritional anemias observed in the country, especially amongst the economically deprived communities.

A wide spectrum of methodologies are now available to assist in the detection and quantitation of the anemic condition due to iron, folate or vitamin B_{12} deficiency. It is to be expected that the combined use of two or more independent indicators would greatly improve diagnostic accuracy. Obviously there is no single test or combination of test that is optimal in all clinical settings. The choice of indicators would depend on a number of factors, including an understanding of the clinical background of the subjects, the volume of blood sample available, and the availability of manpower and equipment. Many of the studies reviewed had to rely only on hemoglobin determination, probably due to unavailability of laboratory facilities.

Studies around the world have shown anemia to be a public health problem of high prevalence in the underdeveloped and tropical areas. It has been adequately shown that iron deficiency is the most common cause of anemia, afflicting large population groups in developing countries as well as in affluent nations. Since pregnant women are particularly vulnerable, the prevalence of anemia in these women has frequently been studied, with rates of 5 to 50% commonly reported for developing countries. In certain countries, high prevalence rates of anemia have also been reported for males who are usually regarded as least at risk. Anemia is also known to be particularly prevalent in pre-school children of economically deprived families.

Prevalence of anemia in Malaysia has been studied in various communities for a long time. Children have been the focus of attention of

numerous studies. Understandably, a wide range of prevalence rates have been observed. Recent studies (after 1970) have shown that rates of 10-40% are commonly encountered for the pre-school and primary school children. Anemia during pregnancy has been recognized as a major cause of maternal mortality in the country as early as in the 1930's. Numerous subsequent studies were carried out, including some recent investigations by the Institute for Medical Research and the Specialist and Reproductive Research Centre. These were however mostly hospital-based studies, specifically the Maternity Hospital, Kuala Lumpur. For obvious reasons of difficulty of obtaining sufficient sample size, community-based studies on pregnant women are scarce. These studies have concentrated on investigating iron deficiency amongst this extremely vulnerable segment of the population, whereas folate and vitamin B_{12}. deficiencies have been investigated only very recently. The anemia problem amongst labourers was investigated in several studies prior to 1960. Recent studies into this group of population are scarce.

The widespread prevalence of anemia has led to questions of its possible effects on the individual as well as on the community as a whole. Although anemia in pregnancy has been a concern to the obstetrician for some years, few data exist to clearly demonstrate that the condition places increased risk to the fetus and mother. There has also been contradictory reports on the possible effects of specific iron, folate and vitamin B_{12} deficiencies of pregnancy. With regards to working efficiency and productivity, objective evidences have been difficult to obtain, and scientific documentation of this has only started to come forth in recent years. Although it has been generally considered that anemic individuals are more susceptible to infections, evidences put forth have been inconclusive and controversies exist. Recently, interest has been focussed on the possible psychological and developmental consequences of iron deficiency anemia. There is however no unequivocal demonstration of an adverse effect of the condition on intellegience, learning, and scholarstic performance. However, it does not mean that the importance of these hemopoietic nutrients are to be taken lightly. Various on-going studies and others in the near future should provide more concrete and well-defined interrelations between the deficiency of these nutrients and the effects out-lined.

Faced with a nutritional problem of such magnitude, and with such possible devastating detrimental effects, governments and the World Health Organization have implemented one or more forms of intervention measures to combat the problem. As a public health measure to deal with the problem of nutritional anemia in the community, these measures would necessarily have to be different from the relatively simple case of treatment of anemia of individuals in the clinics. The various

programs, including therapeutic supplementation, food fortification, and other ancillary measures such as reducing nutrient losses, increasing nutrient absorption and educational campaigns, would probably have to be combined in an integrated approach to achieve the desired results. Some of these programs have been implemented with varying degrees of success in some countries.

This review has shown that the anemia problem has been the concern of several groups of investigators in the country for many years. The problem as it occurs amongst children has been investigated in various isolated studies. Anemia amongst pregnant women, particularly those in the Maternity Hospital in the Capital City has also been studied. A limited number of studies on the labourers and industrial workers have also been reported. The prevalence of the problem nation-wide, especially amongst these vulnerable groups of the population is however still unclear. We therefore need much more information to enable us estimate with more certainty the magnitude of the problem.

Aside from the prevalence of the problem, various aspects of anemia, as it occurs in the country, have remain largely untouched. Hemopoietic nutrient balance, encompassing aspects such as loss, intake, absorption and utilization should be investigated since such factors would depend on the conditions prevailing in the country, and the dietary and food habits of our communities. Our requirements can then be estimated more accurately. From such studies, we could then determine the effects of the anemia condition as encountered by our population, and institute the most suitable intervention measures.

There is therefore much scope for further studies in this field. Contributions from all institutions and research groups, no matter how small that may be, would certainly add towards our understanding of the anemia problem in the country.

BIBLIOGRAPHY

Adams, J.F. and Boddy, K (1968) — Metabolic equilibrium of tracer and natural vitamin B_{12}. *J Lab Clin Med* 72:392-396.

Addison, G.M., Beamish, M.R., Hales, C.N., Hodgkins, M., Jacobs, A. and Llewellin, P. (1972) — An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *J. Clin Pathol* 25:326-329.

Aisen, P. (1982) — Current concepts in iron metabolism. *Clinics in Hemotology* 11(2):242-257.

Albert, M.J., Mathan, V.I. and Baker, S.J. (1980) — Vitamin B_{12} synthesis by human small intestinal bacteria *Nature* 283:781-782.

Alfery, C.P. (1978) — Serum ferritin assay. *CRC Crit Rev Clin Lab Sci* November, pp. 176-208.

Amir Abbas (1973) — Health problems of mothers and children in a rural health survey in Ulu Selangor district. *Proceedings of the National Seminar on the Role of Specialists in Promoting Family Health;* pp. 40-44.

Anderson, A.J.U. (1976a) — Nutrition of Land Dayak children. Final report of a basal nutrition survey, Sarawak pilot Applid Nutrition Project with state relationships and food policy. *Report of the Sarawak Medical Services, Sarawak.*

Anderson, A.J.U. (1976b) — Nutrition of Iban children of the middle Mukah River. *Report of the Sarawak Medical Services, Sarawak.*

Anderson, A.J.U. (1977a) — Nutrition of Iban children of the Lemanak River. *Report of the Sarawak Medical Services, Sarawak.*

Anderson, A.J.U. (1977b) — Nutrition of Malay children of the Sarawak River Delta. *Report of the Sarawak Medical Services, Sarawak.*

Anderson, A.J.U. (1978a) — Nutrition of Iban children of the Sut and Mujong Rivers. *Report of the Sarawak Medical Services, Sarawak.*

Anderson, A.J.U. (1978b) — Subsistence of the Penan in the Mulu area of Sarawak. *Report of the Sarawak Medical Services, Sarawak.*

Andelman, M.B. and Sered, B.R. (1966) — Utilization of dietary iron by term infants: a study of 1048 infants from a low socio-economic population. *Am J Dis Child* 111:45-55.

Anderson, B.B. (1965) — Investigations into the Euglena methods of assay of vitamin B_{12}: the results obtained in human serum and liver using an improved method of assay. *Ph. D. Thesis, U. of London.* Cited in: WHO, 1970; Baker and DeMaeyer, 1979.

Anderson, H.T. and Barkve, H. (1970) — Iron deficiency and muscular work performance. An evaluation of the cardio-respiratory function of iron deficient subjects with and without anemia. *Scan J Clin Lab Invest* 25 (Suppl. 114): 3-62.

Anderson, M.G. and Kelly, A.M. (1981) — Serum ferritin by a rapid and inexpensive ELISA method. *Clin Chim Acta* 116:405-408.

Arbeter, A., Encheverri, L., Franco, D., Munson, D., Velez, H. and Vitale, J.J. (1971) — Nutrition and infection. *Fed Proc* 30:1421-1428.

Aung-Than-Batu, Thein-Than and Thane-Toe (1976) — Iron absorption from Southeast Asian rice-based meals. *Am J Clin Nutr* 29:219-225.

Aykroyd, W.R. and Doughty , J. (1964) — Legumes in human nutrition. *FAO Nutritional Studies No. 19;* Food and Agriculture Organization of the United Nations, Rome.

Bainton, D.F. and Finch, C.A. (1964) — The diagnosis of iron deficiency anemia *Am. J Med 37:62-70*

Baker, H., Herbert, V., Frank, O., Pasher, S., Hunter, M., Wasserman, L.R. and Sobotka, H.A. (1959) — A microbiologic method for detecting folic acid deficiency in man. *Clin Chem* 5:275.

Baker, S.J., Jacob, E., Rajan, K.T. and Swaminathan, S.P. (1962) — Vitamin B_{12} dificiency in pregnancy and the puerperium. *Brit Med J* 1:1658-1661.

Baker, S.J., Kumar, S. and Swaminathan, S.P. (1965) — Excretion of folic acid in bile. *Lancet* 1:685.

Baker, S.J. (1966) — The recognition of vitamin B_{12} and folate deficiency. *NZ Med J* 65(Suppl.):884-891.

Baker, S.J. (1967) — Human Vitamin B_{12} deficiency. *World Rev of Nutr and Dietetics* 8:62-126.

Baker, S.J. (1972) — Tropical Sprue. *Brit Med Bulletin* 28:87-91.

Baker, S.J. (1978) — Nutritional anemia : a major controllable public health problem. *Bulletin of the WHO* 56:659-675.

Baker, S.J. and DeMaeyer, E.M. (1979) — Nutritional anemia: its understanding and control with special reference to the work of the World Health Organization. *Am J Clin Nutr* 32:368-417.

Baker, S.J. (1981) — Nutritional anemias. Part 2. Tropical Asia. *Clinics in Hematology* 10(3):843-871.

Baker, S.J. and Mathan, V.I. (1981) — Evidence regarding the minimal daily requirement of vitamin B_{12} *Am J Clin Nutr* 34:2423-2433.

Ball, E.W. and Giles, C. (1964) — Folic acid and vitamin B_{12} levels in pregnancy and their relation to megaloblastic anemia. *J Clin Pathol* 17:165-174.

Banerji, L., Sood, S.K. and Ramalingaswami, V. (1968) — Geographic pathology of iron deficiency with special reference to India. I. Histochemical quantitation of iron stores in population groups. *Am J Clin Nutr* 21:1139-1148.

Barker, H.A. (1968) — Vitamin B_{12}: biochemical systems.In: The Vitamins: Chemistry, Physiology, Pathway, Methods. Vol. II. Sebrell, W.H.Jr. and Harris, R.S. (eds). Academic Press: N.Y.; pp. 184-212.

Basta, S.S., Soekirman, Karyadi, D. and Scrimshaw, N.S. (1979) — Iron deficiency anemia and the productivity of adult males in Indonesia. *Am J Clin Nutr* 32: 926-925.

Battray, M.J. (1918) — The hemoglobin index and other factors in newly recruited coolies to the Federated Malay States. *Indian Medical Gazette* 53:168-170

Baugh, C.M., Krumdieck, C.L., Baker, H.J. and Butterworth, C.E.Jr. (1971) — Studies on the absorption and metabolism of folic acid. I. Folate absorption in the dog after exposure of isolated intestinal segments to synthetic pteroylpolyglutamates of various chain lenghs. *J Invest* 50: 2009-2021.

Beard, J., Finch, C.A. and Mackler, B. (1981) — Deleterious effects of iron deficiency. In: Nutrition in Health and Disease and International Development. Symposia from the XII International Congress of Nutrition. Harper, A.E. and Davis, G.K. (eds). Alan R. Liss Inc: N.Y.: pp. 305-310

Beaton, G.H. (1974) — Epidemiology of iron deficiency. In: Iron in Biochemistry and Medicine. Jacobs, A. and Worwood, M. (eds). Academic Press : London; pp. 477-528.

Berstein, L.H., Gustein, S., Weiner, S. and Efron, G. (1970) — The absorption and malabsorption of folic acid and its polyglutamates. *Am J Med* 48: 570-579.

Bhaskaram, C. and Reddy, V. (1975) — Cell-mediated immunity in iron and vitamin-deficient children. *Br Med J* 3:522.

Bisseru (1971) — Aspects of soil-transmitted helminths in rural public health. *Pacemaker* 6:44-45.

Bjorn-Rasmussen, E., Hallberg, L., Isaksson, B. and Arvidsson, b. (1974) — Food iron absorption in man. Applications of the two-pool extrinsic tag method to measure heme and nonheme iron absoption from the whole diet. *J Clin Invest* 53:247-255.

Blakley, R.L. (1969) — The biochemistry of folic acid and related pteridines. North Holland Pulb. Co. : Amsterdam

Blumberg, W.E., Eisinger, J., Lamola, A.A. and Zuckerman, D.M. (1977) — Zinc protoporphyrin level in blood determind by a portable hematofluorometer; a screening device for lead poisoning. *J Lab Clin Med* 89:712-723.

Bothwell, T.H., Charlton, R.W., Cook, J.D. and Finch, C.A. (1979) — Clinical evaluation of body iron stores. Iron Metabolism in man. Blackwell Scientific Publications: London.

Bothwell, T.H. and Charlton, R.W. (1981) — Assessment of the iron nutritional status of a population. In: Nutrition in Health and Disease and International Development. Symposia from the XII International Congress of Nutrition. Harper, A.E. and Davis, G.K. (eds). Alan R. Liss Inc: N.Y.; pp. 311-321.

Bothwell, T.H. and Charlton, R.W. (1982) — A general approach to the problems of iron deficiency and iron overload in the population at large. *Seminars in Hematology* 19:54-67.

Bourne, G.H. (1949) — Nutrition work in Malaya under the British Military Administration. *International Review of Vitamin Research* 21(2/3):265-307.

Britt, R.P., Harper, C. and Spray, G.H. (1971) — Megaloblastic anemia among Indians in Britain. *Quarterly J of Med* 40:499-520.

Buckley, R.H. (1975) — Iron deficiency anemia: its relationship to infection susceptibility and host defence. *J Pediatr* 86: 993-995.

Burgess, R.C. and Laidin Alang Musa (1950) — A report on the state of health, the diet and the economic conditions of groups of people in the lower income levels in Malaya. *Institute for Medical Research Report No. 13,* Kuala Lumpur.

Burman, D. (1971) — Iron requirements in infancy. *Br J Hematology* 20:243-247.

Burman, D. (1972) — Hemoglobin levels in normal infants aged 3-24 months and the effect of iron. *Arch Dis Child* 47:261-271.

Burman, D. (1974) — Iron metabolism in infancy and childhood. In : Iron in Biochemistry and Medicine. Jacobs, A. and Worwood M. (eds.). Academic Press : London; pp. 543-562.

Burman, D. (1982) — Iron deficiency in infancy and childhood. *Clinics in Hematology* 11(2):339-351.

Butterworth, C.E.Jr., Santini, R.Jr. and Frommeyer, W.B.Jr. (1963) — The pteroylglutamate components of American diets as determined by chromatographic fractionation. *J Clin Invest* 42: 1928-1939.

Butterworth, C.E.Jr., Baugh, C.M. and Krumdieck, C. (1969) — A study of folate absorption and metabolism in man utilizing carbon-14-labelled polyglutamates synthesized by the solid phase method. *J Clin Invest* 48:1131-1142.

Castle, W.B. (1970) — Current concepts of pernicious anemia. *Am J Med* 48:541-548.

Cavill, I. (1982) — Diagnostic methods. *Clinics in Hematology* 11(2):259-273.

Chanarin, I., MacGibbon, B.M., O'Sullivan, W.J. and Mollin, D.L. (1959) — Folic acid deficiency in pregnancy : the pathogenesis of megaloblastic anemia of pregnancy. *Lancet* 2:634-639.

Chanarin, I., Rothman, D., Perry, J. and Stratfull, D. (1968) — Normal dietary folate, iron and protein intake, with particular reference to pregnancy. *Brit Med J* 2:394-397.

Chanarin, I. (1976) — Investigation and management of megaloblastic anemia. *Clinics in Hematology* 5(3):747-763.

Chanarin, I. (1979) — The megaloblastic anemias. 2nd Edition. Blackwell Scientific Publ.: Oxford.

Chandra, R.K. (1972) — Immunocompetence in undernutrition. *J Pediatr* 81:1194-1200.

Chandra, R.K. (1973) — Reduced bactericidal capacity of polymorphs in iron deficiency. *Arch Dis Child* 48:864-866.

Chandra, R.K. and Saraya, A.K. (1975) — Impaired immonocompetence associated with iron deficiency. *J Pediatr* 86:899-902.

Chappel, R.K. and Janowitz, E.R. (1965) — Health survey on a rural Malay population. FLDA, Sungai Tekam, Pahang. *Med J Malaya* 19:191-200.

Charlton, R.W., Hawkins, D.M., Mavor, W.O. and Bothwell, T.H. (1970) — Hepatic storage iron concentrations in different population groups. *Am J Clin Nutr* 23:358-370.

Charlton, R.W. and Bothwell, T.H. (1982) — Definition, prevalence and prevention of iron deficiency. *Clinics in Hematology* 11(2):309-325.

Chen, P.C.Y., Chan, M.K.C., Teoh, S.T., Bent, B., Yap, S.B., Fong, T., Ong, F.P.T. and Lee, M.C.C. (1981) — A nutrition study of the Interior, West Coast and Kudat Divisions of Sabah. *Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, and Office of the Director of Medical Services, Sabah, Kota Kinabalu.*

Chong, Y.H., Lourdenadin, S., Thean, P.K., Lim, R. and Lopez, C.G. (1968) — Nutritional status during pregnancy. *Far East Med J* 4:214-219.

Chong, Y.H. (1970) — A report on the biochemical assessment of nutritional status of pre-school children in the Applied Nutrition Program pilot area, Telok Datok, Kuala Langat. *Report of the Division of Nutrition, Institute for Medical Research, Kuala Lumpur.*

Chong, Y.H., McKay, D.A. and Lim, R.K.H. (1972) — Some results of recent nutrition surveys in West Malaysia (with emphasis on nutritional anthropometry and biochemistry). *Bulletin of the Public Health Society* 6:55-61.

Chong, Y.H. (1974) — Biochemical assessment of the nutritional status of pre-school children in Kuala Trengganu. *Med J Malaysia* 28:213-220

Chong, Y.H., Lim, R.K.H., Foo, L.C., Tee, E.S., Hanis, H. and Kandiah, M. (1979) — Highlights and recommendations : Kelantan nutrition survey. *Report of the Division of Human Nutrition, Institute for Medical Research, Kuala Lumpur.*

Chong, Y.H., Lim, R.K.H., Foo, L.C., Tee, E.S., Ng, T.K.W., Hanis, H., Kandiah, M. and Tan, A.T. (1981) — Highlights and conclusions of a nutrition survey conducted on two padi-growing kampongs, Padang Endau, Mersing, Johore. *Report of the Division of Human Nutrition, Institute for Medical Research, Kuala Lumpur.*

Chong, Y.H., Lim, R.K.H., Foo, L.C., Tee, E.S., Ng, T.K.W., Hanis, H., Kandiah, M. and Teo, P.H. (1982) — Report on the status of community nutrition of four poverty kampungs, Baling, Kedah. *Report of the Division of Human Nutrition, Institute for Medical Research, Kuala Lumpur.*

Chong, Y.H., Tee, E.S., Ng, T.K.W., Kandiah, M., Siti M. Shahid, R.Hanis Hussein and Teo, P.H. (1983) — Report of the status of community nutrition of six poverty kampungs, Perak Tengah, September 1983. *Report of the Division of Human Nutrition, Institute for Medical Research, Kuala Lumpur.*

Chong, Y.H., Tee, E.S., NG, T.K.W., Kandiah, M., R. Hanis Hussein, Teo, P.H. and Siti M. Shahid (1984) — Status of community nutrition of poverty kampungs. *Division of Human Nutrition, Institute for Medical Research Bulletin No. 22, Kuala Lumpur.*

Chung, A.S.M., Pearson, W.N., Darby, W.J., Miller, O.N. and Goldsmith, G.A. (1961) — Folic acid, vitamin B_6, pantothenic acid and vitamin B_{12} in human dietaries. *Am J Clin Nutr* 9:573-582.

Cifuentes, E. and Viteri, F. (1972) — Physical fitness, iron deficiency and anemia in agricultural labourers of Central America. *Fed Proc* 31:719.

Cole, S.K., Billewicz, W.Z. and Thomson, A.M. (1971) — Sources of variation in menstrual blood loss. *J Obstet Gynecol British Commonwealth* 78: 933-939.

Colman, N., Barker, M., Green, R. and Metz, J. (1974) — Prevention of folate deficiency in pregnancy by food fortification. *Am J Clin Nutr* 27:339-344.

Colman, N., Green, R. and Metz, J. (1975) - Prevention of folate deficiency in pregnancy by food fortification. II. Absorption of folic acid from fortified staple foods. *Am J Clin Nutr* 28:459-464.

Colman, N., Larsen, J.V., Barker, M., Barker, A.E., Green, R. and Metz, J. (1975) — Prevention of folate deficiency by food fortification. III. Effect in pregnant subjects by varying amounts of added folic acid. *Am J Clin Nutr* 28:465-470.

Cook, J.D., Alvarado, J., Gutnisky, A., Jamra, M., Labardini. J., Layrisse. M., Linares, J., Loria, A., Maspes, V., Restrepo, A., Reynafarje, C., Sanchez-Medal, L., Velez, H. and Viteri, F. (1971) — Nutritional deficiency and anemia in Latin America: A collaborative study. *Blood* 38:591-603

Cook, J.D., Layrisse, M., Martinez-Torres, C., Walker, W., Monsen, E. and Finch, C.A. (1972) — Food iron absorption measured by an extrinsic tag. *J Clin Invest* 51:805-815.

Cook, J.D., Lipschitz, D.A. and Miles, L.E.M. (1974) — Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 27:681-687.

Cook, J.D., Finch, C.A. and Smith, N.J. (1976) — Evaluation of the iron status of a population. *Blood* 48:449-445.

Cook, J.D., and Finch, C.A. (1979) — Assessing iron status of a population. *Am J Clin Nutr* 32:2115-2119.

Cook, J.D. (ed.) (1980) — Iron. *Methods in Hemotology, Volume 1,* Churchill Livingstone.

Cook, J.D., Morck, T.A., Skikne, B.S., Lynch, S.R. (1981) — Biochemical determinants of iron absorption. In: Nutrition in Health and Diseases and International Development. Symposia from the XII International Congress of Nutrition. Harper, A.E. and Davis, G.K. (eds). Alan R Liss Inc : N.Y.; pp. 323-331.

Cook, J.D. (1982) — Clinical evaluation of iron deficiency. *Seminars in Hematology* 19(1):6-18.

Coovadia, H.M., Parent, M.A., Loening, W.E.K, Wesley, A., Burgess, B., Hallet, F., Brain, P., Grace, J., Naidoo, J., Smythe, P.M. and Vos,

G.H. (1974) — An evaluation of factors associated with the depression of immunity in malnutrition and in measles. *Am J Clin Nutr* 27:665-669.

Corcino, J.J., Waxman, S. and Herbert, V. (1970) — Absorption and malabsorption of vitamin B_{12}. *Am J Med* 48:562-569.

Corke, W.H. and Bush, L.M. (1980) — An investigation of some cases of anemia in Tamil women. *Malayan Med J* 5:129-135.

Council on Food and Nutrition, Committee on Iron Deficiency (1968) — Iron deficiency in the United States. *J Am Med Assoc* 203:407-414.

Crichton, R.R.(1975) — Ferritin: structure, function and role in intracellular iron metabolism. In: Iron Metabolism and its Disorders. Kief, H. (ed.). Excerpta Medica: Amsterdam; pp. 81-89.

Dallman, P.R., George, D.B., Allen, C.M. and Shinefield, H.R. (1978) — Hemoglobin concentration in white, black, and oriental children: is there a need for separate criteria in screening for anemia ? *Am J Clin Nutr* 31:377-380.

Dallman, P.R. and Siimes, M.A. (1979) — Percentile curves for hemoglobin and red cell volume in infancy and childhood. *J Pediatr* 94:26-31.

Dallman, P.R., Siimes, M.A, and Stekel, A. (1980) — Iron deficiency in infancy and childhood. *Am J Clin Nutr* 33:86-118.

Dallman, P.R. (1981) — Nutritional anemias: relationships to genetic and pharmacological factors. In: Nutrition in the 1980s : Constraints on Our Knowledge. Selvey, N. and White, P.L. (eds). Alan R. Liss Inc: N.Y.; pp. 87-96.

Dallman, P.R. (1982) — Manifestations of iron deficiency. *Seminars in Hematology* 19(1):19-30.

Das, K.C. and Hoffbrand, A.V. (1970) — Lymphocyte transformation in megaloblastic anemia : morphology and DNA synthesis. *Br J Hemat* 19:459-468.

Das, K.C. and Herbert, V. (1976) — Vitamin B_{12} — folate interrelations. *Clinics in Hematology* 5(3):697-725.

Davies, C.T.M. (1973) — Relationship of maximum aerobic power output to productivity and absenteeism of East African sugar cane workers. *Brit J Industr Med* 30:146-154.

Davies, C.T.M., Chukweumeka, A.C. and van Haaren, J.P.M. (1973) — Iron deficiency anemia : its effects on maximum aerobic power and response to exercise in African males aged 17-40 years. *Clin Sci* 44: 555-562.

Davies, C.T.M. and van Haaren, J.P.M. (1973) — Effect of treatment on physiological responses to exercise in East African industrial workers with iron deficiency anemia. *Brit J Industr Med* 30:335-340.

Davis, R.E., Stenhouse, N.S. and Woodliff, H.J. (1969) — Serum folate levels in pregnancy. *Med J Aust* 1:52-53.

Davis, C. (1970) — The relationship of heavy infections of *Ascaris lumbricoides* to malnutrition. *University of California International Centre for Medical Research and Training Annual Progress Report,* pp. 97-106.

DeMaeyer, E.M. (1981) — The role of WHO in the control of nutritional anemia. In: Nutrition in Health and Disease and International Development. Symposia from the XII International Congress of Nutrition. Harper, A.E. and Davis, G.K. (eds). Alan R.Liss Inc : N.Y. pp. 363-370.

Diamond, L.K. (1970) — Iron deficiency in history and practice. In: Iron Nutrition in Infancy. Report of the 62nd Ross Conference on Pediatric Research on Pediatric Research. Ross Laboratories: Ohio; pp 10-11.

Eagland, R.D. (1952) — A report on anemia in estate labourers, Malaya. *Med J Malaya* 7:36-38.

Edelman, R., Suskind, R., Olson, R.E. and Sirisinha, S. (1973) — Mechanisms of defective delayed cutaneous hypersensitivity in children with protein-calorie malnutrition. *Lancet* 1:506-508.

Edgerton, V.R., Gardner, G.W., Ohira, Y., Gunawardena, K.A. and Senewiratne, B. (1979) — Iron deficiency anemia and its effects on worker productivity and activity patterns. *Br Med J* 2:1546-1549.

Ekblom, B., Goldberg, A.N. and Gullbring, B. (1972) — Response to exercise after blood loss and reinfusion. *J Appl Physiol* 33:175-180.

Elwood, P.C. (1970) — Some epidemiological aspects of iron deficiency relevant to its evaluation. *Proc Roy Soc Med* 63:1230-1232.

Fairbanks, V.F., Fahey, J.L. and Beutler, E. (1971) — Clinical Disorders of Iron Metabolism. 2nd edition. Grune & Stratton.

Fairbanks, V.F. (1978) — Nutrient deficiencies in man : Iron. In: CRC Handbook Series in Nutrition and Food. Volume III. Effect of Nutrient Deficiencies in Man. Rechcigl, M. (ed). CRC Press; pp. 183-221.

Fielding, J. (1975) — Clinical symptoms and syndromes of iron deficiency. In: Iron Metabolism and its Disorders, Kief, H. (ed). Experpta Medica Amsterdam; pp. 126-134.

Fielding, J. (1980) — Serum iron and binding capacity. In: Iron. Methods in Hematology, Volume 1. Cook, J.D. (ed). Churchill Livingstone; pp. 15-43.

Finch, C.A. (1970) — Diagnostic value of different methods to detect iron deficiency. In: Iron Deficiency. Hallberg, L., Harwerth, H.G. and Vannotti, A. (eds). Academic Press: N.Y.; pp. 409-416.

Finch, C.A. and Huebers, H. (1982) — Perspectives in iron metabolism. *New Engl J Med* 306:1520-1528.

Fleming, A.F. (1970) — Seasonal incidence of anemia in pregnancy in Ibadan. *Am J Clin Nutr* 23:224-230.

Fleming, A.F. (1982) — Iron deficiency in the tropics. *Clinics in Hematology* 11(2):365-388.

Fletcher, J., Mather, J., Lewis, M.J. and Whiting, G. (1975) — Mouth lesions in iron-deficient anemia : relationship to *Candida albicans* in saliva and to impairment of lymphocyte transformation. *J. Infect Dis* 131:44-50.

Fong, T.P., Okafor, L.A., Thomas, W. Jr. and Westerman, M.P. (1977) — Stainable iron in aspirated and needle-biopsy specimens of marrow: a source of error. *Am J Hematol* 2:47-51.

Foster, R.M. (1968) — The seasonal incidence of megaloblastic anemia of Mombasa. *East Afr Med J* 45:673-676.

Garby, L., Irnell, L., Werner, I. (1969a) — Iron deficiency in women of fertile age in a Swedish community. III. Estimation of prevalence based on responses to iron supplementation. *Acta Med Scand* 185:113-117.

Garby, L., Irnell, L., Werner, I. (1969b) – Iron deficiency in women of fertile age in a Swedish community. II. Efficiency of several laboratory tests to predict the response to iron supplementation. *Acta Med Scand* 185:107-111.

Gardner, G.W., Edgerton, V.R., Senewiratne, B., Barnard, R.J. and Ohira, Y. (1977) — Physical work capacity and metabolic stress in subjects with deficiency anemia. *Am J Clin Nutr* 30:910-917.

Garn, S.M., Smith, N.J. and Clark, D.C. (1975a) — Life long differences in hemoglobin levels between blacks and whites. *J Natl Med Assoc* 67:91. Cited in: Dallman, Siimes and Stekel (1980).

Garn, S.M., Smith, N.J. and Clark, D.C. (1975b) — The magnitude and implications of apparent race differences in hemoglobin values. *Am J Clin Nutr* 28:563-568.

Gatenby, P.B.B. and Lillie, E.W. (1960) — Clinical analysis of 100 cases of severe megaloblastic anemia of pregnancy. *Br Med J* 2: 111-1114

George, E., Adeeb, N. and Ahmad, J. (1980) — Iron stores in pregnancy. *Med J Malaysia* 35(2):129-130.

George, J. and Ow-Yang, C.K. (1982) — Prevalence of soil transmitted helmiths in school children in the Federal Territory of Malaysia. *Med J Malaysia 37:35-39.*

Goltner, E. (1975) — Iron requirement and deficiency in menstruating and pregnant women. In: Iron metabolism and its Disorders. Keif, H. (ed). Excerpta Medica: Amsterdam; pp. 159-167.

Graitcer, P.L., Goldsby, J.B. and Nichaman, M.Z. (1981) — Hemoglobin and hematocrits : are they equally sensitive in detecting anemias? *Am J Clin Nutr* 34: 61-64.

Grasbeck, R., Nyberg, W. and Reizenstein, P. (1958) — Biliary and fecal vitamin B_{12} excretion in man: An isotope study. *Proc Soc Exptl Biol Med* 97:780-784.

Grasbeck, R. (1960) — Physiology and pathology of vitamin B_{12} absorption, distribution and excretion. *Advan Clin Chem* 3:299-366.

Green, R., Charlton, R., Seftel, H., Bothwell, T., Mayet, F., Adams, B., Finch, C. and Layrisse, M. (1968) — Body iron excretion in man: A collaborative study. *Am J Med* 45:336-353.

Gross, R.L., Reid, J.V.O., Newberne, P.M., Burgess, B., Marston, R. and Hift, W. (1975) — Depressed cell-mediated immunity in megaloblastic anemia due to folic acid deficiency. *Am J Clin Nutr* 28:225-232.

Grossowicz, N., Rachmilewitz, M. and Izak, G. (1972) — Absorption of pteroylglutamate and dietary folates in man. *Am J Clin Nutr* 25:1135-1139.

Grossowicz, N., Rachmilewitz, M, and Izak, G. (1975) — Utilization of yeast polyglutamate folates in man. *Proc Soc Exptl Biol Med* 150:77-79.

Hallberg, L., Hogdahl, A.M., Nilsson, L. and Rybo, G. (1966) — Menstrual blood loss : a population study. *Acta Obstet Gynecol Scand* 45:320-351.

Hallberg, L. and Bjorn-Rasmussen, E. (1972) — Determination of iron absorption from whole diet. A new two-pool model using two radio-iron isotopes given as heme and non-heme iron. *Scand J Hematol* 9:193-197.

Hallberg, L. (1981) — Bioavailability of iron from different meals. In: Nutrition in Health and Disease and International Development. Symposia from the XII International Congress of Nutrition. Harper, A.E. and Davis, G.K. (eds). Alan R. Liss Inc: N.Y.; pr. 333-343.

Hallberg, L. (1982) — Iron nutrition and food iron fortification. *Seminar in Hematology* 19(1):31-41.

Halliday, J.W. and Powell, L.W. (1982) — Iron overload. *Seminars in Hematology* 19:42-53.

Halsted, J.A., Lewis, P.M., Hvolboll, E.E., Gasster, M. and Swendseid, M.E. (1956) — An evaluation of the fecal recovery method for determining intestinal absorption of cobalt ^{60} labelled vitamin B 12. *J. Lab Clin Med* 48:92.

Hansen, H.A. and Weinfeld, A. (1962) — Metabolic effects and diagnostic value of small doses of folic acid and B_{12} in megaloblastic anemia. *Acta Med Scand* 172:427-443.

Hansen, H.A. (1967) — On the diagnosis of folic acid deficiency. *Acta Obstet Gynecol Scand* 46 (Suppl): 13-37

Hansen, H.A. and Rybo, G. (1967) — Folic acid dosage in prophylactic treatment during pregnancy. *Acta Obstet Gynecol Scand* 46 (Suppl): 107-112.

Harrison, K.A. (1982) - Anemia, malaria and sickle cell disease. *Clinics in Obstet and Gynecol* 9(3):445-477.

Harrison, P.M., Hoare, R.J., Hoy, T.G. and Macara, I.G. (1974) — Ferritin and hemosiderin : structure and function. In: Iron in Biochemistry and Medicine. Jacobs, A. and Worwood, M. (eds). Academic Press: London; pp. 73-114.

Hawkins, W.W. (1964) — Iron, copper and cobalt. In: Nutrition : A Comprehensive Treatise. Beaton, G.H. and McHenry, E.W. (eds). Academic Press: N.Y.; pp. 309-472.

Helmeyer, L. and Harwerth, H.G. (1970) — Clinical manifestations of iron deficiency. In: Iron Deficiency. Hallberg, L., Harwerth, H.G. and Vannotti, A. (eds). Academic Press: N.Y.; pp. 375-382.

Henrich, H.C. (1970) — Intestinal iron absorption in man: methods of measurement, dose relationship, diagnostic and therapeutic aplications. In: Iron Deficiency. Hallberg, L., Harwerth, H.G. and Vannotti, A. (eds). Academic Press: N.Y.; pp. 213-294.

Herpner, G.W., Booth, C.C., Cowan, J., Hoffbrand, A.V. and Mollin, D.L. (1968) — Absorption of crystalline folic acid in man. *Lancet* 2:302-306.

Herbert, V. (1962a) — Minimal daily adult folate requirement. *Arch Intern Med* 110:649-652.

Herbert, V., Baker, H., Frank, O., Pasher, I., Sabotka, H. and Wasserman, L.R. (1960) — The measurement of folic acid activity in serum : a diagnotic aid in the differentiation of the megaloblastic anemias. *Blood* 15: 228-235.

Herbert, V. (1926b) — Experimental nutritional folate deficiency in man. *Trans Assoc Am Physns* 75:307. Cited in: Chanarin, 1979.

Herbert, V. (1966) — Aseptic addition method for *Lactobacillus casei* assay of folate activity in human serum. *J Clin Pathol* 19:12-16.

Herbert, V. and Bertino, J.R. (1967) — Folic acid. In: The Vitamins. Volume VII. Gyorgy, P. and Pearson, W.N. (eds). Academic Press : N.Y., pp. 143-169.

Hershko, C., Bar-Or, D., Gaziel, Y., Naparstek, E., Konijin, A.M., Grosswicz, N., Kaufman, N. and Izak, G. (1981) — Diagnosis of iron deficiency anemia in a rural population of children. Relative usefulness of serum ferritin, red cell protopophyrin, red cell indices, and transferrin saturation determinations. *Am J Clin Nutr* 34:1600-1610.

Heyssel, R.M., Bozian, R.C., Darby, W.J. and Bell, M.C. (1966) — Vitamin B_{12} turnover in man: the assimilation of vitamin B_{12} from natural foodstuff by man and estimates of minimal daily requirements. *Am J Clin Nutr* 18:176-184.

Higashi, O., Sato, Y., Takamatsu, H. and Oyama, H. (1967) — Mean cellular peroxidase (MCP) of leukocytes in iron deficiency anemia. *Tohoku J Exp Med* 93: 105. Cited in: Pearson and Robinson, 1976.

Hoffbrand, A.V., Newcombe, B.F.A. and Mollin, D.L. (1966) — Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency. *J Clin Pathol* 19:17-28.

Hoffbrand, A.V., Necheles, T.F., Moldonado, N., Horta, E. and Santini, R. (1969) — Malabsorption of folate polyglutamates in tropical sprue. *Brit Med J* 2:543-547.

Hoffbrand, A.V. (1978) — Effects of folate deficiency in man. In: CRC Handbook Series in Nutrition and Food. Volume III. Effect of Nutrient Deficiencies in man. Rechcigl, M. (ed). CRC Press; pp. 55-68.

Hoppner, K., Lampi, B. and Perrin, D.E. (1972) — The free and total folate activity in foods available on the Canadian marker. *J Inst Canad Sci Technol Aliment* 5:60. Cited in: Malin, 1975.

Hurdle, A.D.F. (1968a) — An assessment of the folate intake of elderly patients in hospitals. *Med J Aust* 55: 101-104.

Hurdle, A.D.F. (1968b) — The influence of hospital food on the folic acid stauts of long-stay elderly patients. *Med J Aust* 55:104-111.

ICNND (Interdepartmental Committee On Nutrition for National Defence) (1963) — Federation of Malaya nutrition survey (September — Oktober (1962). *ICNND Report, United States Government Printing Office.*

Jacobs, A. (1974) — Erythropoiesis and iron deficiency anemia. In: Iron in Biochemistry and Medicine. Jacobs, A. and Worwood, M. (eds). Academic Press: London; pp. 405-436.

Jacobs, A. and Joynson, D.H.M. (1974) — Lymphocyte function and iron deficiency anemia. *Lancet* 2:844.

Jacobs, A. and Worwood, M. (1975) — Ferritin in serum: clinical and biochemical implications. *New Engl J Med* 292:951-956.

Jadhav, M., Webb, J.K.G., Vaishnava, S. and Baker, S.J. (1962) — Vitamin B_{12} deficiency in Indian infants: a clinical syndrome. *Lancet* 2:903-907.

Jaffar Ali, Khalid Hassan and Hamid Arshat (1981) — Suggestion of an active transport of iron to the fetus in human pregnancy and its dependence on maternal serum iron levels. *Med J Malaysia* 36(4): 215-219.

Jaffar Ali, Khalid Hassan and Hamid Arshat (1982) — Maternal and cord folate and vitamin B_{12} levels in Malaysians at parturition. *Med J Malaysia* 37(2):160-164

James, J.A. and Combes, M. (1960) — Iron deficiency in the premature infant. *Pediatrics* 26:368-374.

Jeejeebhoy, K.N., Desai, H.G., Borkar, A.V., Deshpande, V. and Pathare, S.M. (1968) — Tropical malabsorption syndrom in West India. *Am J Clin Nutr* 21:994-1006.

Joske, R.A. (1963) — The vitamin B_{12} content of human liver tissue obtained by aspiration biopsy. *Gut* 4:231-235.

Joynson, D.H.M., Jacobs, A., Walker, D.M. and Dolby, A.E. (1972) — Defect of cell-mediated immunity in patients with iron deficiency anemia. *Lancet* 2:1058-1059

Judisch, J.M., Naiman, J.L. and Oski, F.A. (1966) — The fallacy of the fat iron-deficient child. *Pediatrics* 37:987-990.

Kandiah, M. and Lim, J.B. (1976) — Report on the health status of a group of traditional Malay villages in Ulu Jempol. *Institute for Medical Research Bulletin Number 17, Rural Health Research Study Number 1,* Kuala Lumpur.

Kandiah, N. and Lim, J.B. (1977) — Nutritional status in a rural estate community. *Med J Malaysia* 31:270-276.

Kandiah, N. and Tan, D. (1977) — Report on the health status of settlers in an SLDA Scheme in Ulu Rening. *Institute for Medical Research Bulletin Number 18, Rural Health Research Study Number 2,* Kuala Lumpur

Kandiah, M., Lee, M., Ng, T.K.W. and Chong, Y.H. (1984) — Malnutrition in malaria endamic villages of Bengkoka Peninsula, Sabah. *J Trop Pediatr* 30:23-29.

Kapadia, C.R., Mathan, V.I. and Baker, S.J. (1976) — Free intrinsic factor in the small intestine of man. *Gastroenterology* 70:704-706.

Khalid Hassan, Jaffar Ali, Hamid Arshat and Noor Laily Abu Bakar (1984) — personal communications.

Kief, H. (1975) (Editor) — Iron Metabolism and its Disorders. **Proceedings** of the Third Workshop Conference Hoechst, Schloss Reisensburg. Excerpta Medica.

Kinzie, J.D., Kinzie, K. and Tyas, J. (1966) — A comparative health survey among two groups of Malayan aborigines. *Med J Malaya* 21:135-139.

Klee, G.G., Fairbanks, V.F., Pierre, R.V. and O'Sullivan, M.B. (1976) — Routine erythrocyte measurements in diagnosis of iron deficiency anemia and thalassemia minor. *Am J Clin Pathol* 66:870-877.

Klipstein, F.A. and Baker, S.J. (1970) — Regarding the definition of tropical sprue. *Gastroenterology* 58:717-721.

Koerper, M.A. and Dallman, P.R. (1977) — Serum iron concentration and tranferrin saturation in the diagnosis of iron deficiency in children : normal developmental changes. *J Pediatrics* 91:870-874.

Krause, J.R., Brubaker, D. and Kaplan, S. (1979) — Comparison of stainable iron in aspirated and needle-point biopsy specimens of bone marrow. *Am J Clin Pathol* 72:68-70.

Kulapongs, P., Vithayasai, V., Suskind, R. and Olson, R.E. (1974) — Cell-mediated immunity and phagocytosis and killing function in children with severe iron deficiency anemia. *Lancet* 2:689-691.

Labbe, R.F. and Finch, C.A. (1980) — Erythrocyte protoporphyrin : aplication in the diagnosis of iron deficiency. In: Iron. Methods in Hematology. Volume 1. Churchill Livingstone. pp. 44-58.

Lamprell, B.A. and Cheek, E. (1952) — Anemia in southern Indians employed on Malayan plantations (A review of causes and methods of treatment in an exceptionally anemic population). *Med J Malaya* 7: 107-114.

Langer, E.E., Haining, R.G., Labbe, R.F., Jacobs, P., Crosby E.F. and Finch, C.A. (1972) — Erythrocyte protoporphyrin. *Blood* 40:112-128.

Lanzkowsky, P. (1961) — The influence of maternal iron deficiency anemia on the hemoglobin of the infant. *Arch Dis Child* 36:205-209.

Law, D.K., Dudrick, S.J. and Abdou, N.I. (1973) — Immunocompetence of patients with protein-calorie malnutrition : the effects of nutritional repletion. *Ann Intern Med* 79:545-550.

Layrisse, M. and Roche, M. (1964) — The relationship between anemia and hookworm infection : results of surveys of rural Venezuelan population. *Am J Hyg* 79:279-301.

Layrisse, M., Aparcado, L., Martinez-Toores, C. and Roche, M. (1967) — Blood loss due to infection with *Trichuris trichiura*. *Am J Trop Med Hyg* 16:613-619.

Layrisse, M., Martinez-Torres, C. and Roche, M. (1968) — Effect of interaction of various foods on iron absorption. *Am J Clin Nutr* 21:1175-1183.

Lyrisse, M. and Martinez-Torres, C. (1972) — Model for measuring dietary absorption of heme iron : test with a complete meal. *Am J Clin Nutr* 25:401-411.

Lyrisse, M. (1975) — Dietary iron absorption. In: Iron Metabolism and its Disorders. Kief, H. (ed). Excerpta Medica; Amsterdam; pp. 25-33.

Lyrisse, M., Roche, M. and Baker, S.J. (1976) — Nutritional anemias. In: Nutrition in Preventive Medicine. The Major Deficiency Syndromes, Epidemiology and Approaches to Control. Beaton, G.H. and Bengoa, J.M. (eds). *WHO Monograph Series No. 62,* Geneva; pp. 55-82.

Lyrisse, M. (1980) — Iron intake and iron absorption. In: Nutrition and Food Science : Present Knowledge and Utilization. Volume 3. Nutritional Biochemistry and Pathology. Santos, W., Lopes, N., Barbosa, J.J., Chaves, D. and Valente, J.C. (eds). Plenum Press: N.Y.; pp. 123-129.

Leibel, R.L., Greenfield, D.B. and Pollitt, E. (1979) — Iron deficiency : Behaviour and Brain Biochemistry. In: Nutrition — Pre-and Postnatal Development. Winick, M. (ed). Chapter 13. Plenum Press: N.Y. Cited in: Burman, 1982.

Lie-Injo, L.E. and Virik, H.K. (1966) — Anemias in children in Malaya. *Trans Royal Soc Trop Med Hyg* 60:53-63.

Lipschitz, D.A., Cook, J.D. and Finch, C.A. (1974) — A clinical evaluation of serum ferritin as an index of iron stores. *New Engl J Med* 290:1213-1216.

Llewellyn-Jones, D. (1965) — Severe anemia in pregnancy as seen in Kuala Lumpur, Malaysia. *Aust NZ J Obstet Gynecol* 5:191-197.

Lo, E.K.C., Varughese, J., Ghouse, A. and Noor, M. (1979) — Helminthiases in Peninsular Malaysia: Prevalence and density of infestation of hookworm, *ascaris* and *trichuris* in rural school children. *Med J Malaysia* 34: 95-99.

Lourdenadin, S. (1964) — Pattern of anemia and its effects on pregnant women in Malaya. *Med J Malaya* 19:87-93.

Lourdenadin, S. (1969) — Hazards of anemia in pregnancy in Malaysia. *Intl J Gynecol Obstet* 7:234-242.

Lovric, V.A. (1970) — Normal hematological values in children aged 6-36 months and socio -medical implications. *Med J Aust* 2:336-370.

Luken, J.N. (1975) — Iron deficiency and infections : fact or fable. *Am J Dis Child* 129:160-162.

Macdougall, L.G., Anderson, R., McNab, G.M. and Katz, J. (1975) — The immune response in iron deficient children : impaired cellular defence mechanisms with altered humoral components. *J Pediatr* 86:833-843.

MacGregor, M.W. (1963) — Maternal anemia as a factor in prematurity and perinatal mortality. *Scot Med J* 8:134.

Mackay, H.M. (1928) — Anemia in infancy : its prevalence and prevention. *Arch Dis Child* 3:117-147. Cited in: Luken, 1975; Pearson and Robinson, 1976.

Mackay, H.M. (1931) — Nutritional anemia in infancy : with special reference to iron deficiency. *Med Res Council (G.B) Spec Rep Ser No. 157,* London; pp. 1-125. Cited in: Fairbanks, 1978.

Malin, J.D. (1975) — Folic acid. *Wld Rev Nutr Diet* 21:198-223.

Margo, G., Barker, M., Fernandes-Costa, F., Colman, N., Green, R. and Metz, J. (1975) — Prevention of folate deficiency by food fortification. VII. The use of bread as a vehicle for folate supplementation. *Am J Clin Nutr* 28:761-763.

Masawe, A.E.J. and Nsanzumuhire, H. (1973) — Growth of bacteria *in vitro* in blood from patients with severe iron deficiency anemia and from patients with sickle cell anemia. *Am J Clin Pathol* 59:706-711.

Masawe; A.E.J., Muindi, J.M. and Swai, B.R, (1974) — Infection in iron deficiency and other types of anemia in the tropics. *Lancet* 2:314-317.

Masawe, A.E.J., (1981) — Nutritional anemias. Part 1. Tropical Africa. *Clinics in Hematology* 10(3):815-841.

McCance, R.A. and Widdowson, E.M. (1967) — The composition of foods. *Med Res Council Spec Rep Ser No. 297,* London.

Metz, J. (1963) — Folates in megaloblastic anemia. *Bull Wld Hlth Org* 28:517-529.

Mollin, D.L. and Ross, G.I.M. (1952) — The vitamin B_{12} concentrations of serum and urine of normals and patients with megaloblastic anemias and other diseases. *J Clin Pathol* 5:129-139.

Mollin, D.L., Anderson, B.B. and Burman, J.F. (1976) — The serum vitamin B_{12} levels : its assay and significance. *Clinics in Hematology* 5(3):521-546.

Moore, H.W. and Folkers, K. (1968) — Vitamin B_{12} : chemistry. In: The Vitamins : Chemistry, Physiology, Pathology, Methods. Volume II. Sebrell, W.H. Jr and Harris, R.S. (eds). Academic Press: N.Y.; pp. 121-139.

Moore, C.V. (1970) — Concluding statement. In: Iron deficiency. Hallberg, L., Harwerth, H.G. and Vannotti, A. (eds). Academic Press: N.Y.' pp. 625-628.

Morgan, E.H. (1974) — Transferrin and transferrin iron. In: Iron in Biochemistry and Medicine. Jacobs, A. and Worwood, M. (eds). Academic Press: London; pp. 29-71.

Murray, M.J., Murray, A.B., Murray, N.J., and Murray, M.B., (1978) — The effect of iron status of Nigerian mothers on that of their infants at birth and 6 months, and on the concentration of iron in breast milk. *Br J Nutr* 39:627-630.

Ng, T.K.W. (1978) — Biochemical assessment of the nutritional status of Shah Alam industrial workers. *Report of the Division of Human Nutrition, Institute for Medical Research, Kuala Lumpur;* mimeographed.

Nixon, P.F. and Bertino, J.R. (1970) — Interrelationships of vitamin B_{12} and folate in man. *Am J Med* 48:555-561.

Ong, H.C. (1973) — Hematological values in pregnancy in Orang Asli (aboriginal) women. *Med j Malaya* 27:240-25.

Oski, F.A. and Honig, A.S. (1978) — The effects of theraphy on the developmental scores of iron-deficient infants. *J Pediatr* 92:21-25.

Pallister, R.A. (1934) — Some observations on tropical anemias. *Malayan Med J* 9:43-50.

Patwardhan, V.N. (1966) — Nutritional anemias : WHO research program. Early developments and progress report of collaborative studies. *Am J Clin Nutr* 19:63-71.

Pearson, H.A. and Robinson, J.E. (1976) — The role of iron in host resistance. *Adv in Pediatr* 23:1-33.

Perry, J. and Chanarin, I. (1968) — Absorption and utilization of polyglutamyl forms of folate in man. *Brit Med J* 4:546-549.

Perry, J. and Chanarin, I. (1972) — Observations on folate absorption with particular reference to folate polyglumates and possible inhibitors to its absorption. *Gut* 13:544-550.

Pilon, V.A., Howanitz, P.J., Howanitz, J.H. and Domres, N. (1981) — Day-to-day variation in serum ferrition concentration in healthy subjects. *Clin Chem* 27: 78-82.

Piomelli, S., Brickman, A. and Carlos, E. (1976) — Rapid diagnosis of iron deficiency by measurement of free erythrocyte porphyrins and hemoglobin : the FEP/hemoglobin ratio. *Pediatrics* 57:136-141.

Pollitt, E. and Leibel, R.L. (1976) — Iron deficiency and behaviour. *J Pediatr* 88:372-381.

Pratt, J.M. (1972) — Inorganic chemistry of vitamin B_{12}. Academic Press: London; pp. 1-43.

Reed, J.G. (1940) — Maternal mortality among South Indian estate women. *J Malaya Branch, Brit Med Assoc* 4:13-32.

Reed, J.G. (1947) — Notes on some estate vital statistics. *Med J Malaya* 2:20-26.

Rios, E., Lipschitz, D.A., Cook, J.D. and Smith, N.J. (1975) — Relationship of maternal and infant iron stores as assessed by determination of plasma ferrittin. *Pediatrics* 55:694-699.

Roche, M. and Layrisse, M. (1966) — The nature and cause of "hookworm anemia". *Am J Trop Med Hyg* 15:1031-1100.

Romine, M.K. (1960) — The folic acid activity of human livers as measured with *Lactobacillus casei. J Vit* 6:196. Cited in : Baker and DeMaeyer, 1979.

Rosenberg, I.H. and Goodwin, H.A. (1971) — The digestion and absortion of dietary folate. *Gastroenterology* 60:445-463.

Rosenberg, I.H. (1976) — Absorption and malabsorption of folates. *Clinics in Hematology* 5(3):589-618.

Roszkowski, I., Wojcicka, J. and Zaleska, K. (1966) — Serum iron deficiency during the third trimester of pregnancy : maternal complications and fate of the neonate. *Obstet Gynecol* 28:820-825.

Rothenberg, S.P. and Da Costa, M. (1976) — Folate binding proteins and radioassay for folate. *Clinics in Hematology* 5(3):569-587.

Rotenberg, S.P. and Cotter, R, (1978) — Nutrient deficiencies in man : vitamin B_{12} In: CRC Handbook Series in Nutrition and Food. Volume III. Effect of Nutrient Deficiencies in Man. Rechcigl, M. (ed). CRC Press; PP. 69-88.

Rothman, D. (1970) — Folic acid in pregnancy. *Am J Obstet Gynecol* 108:149-175.

Royston, E. (1982) — The prevalence of nutritional anemia in women in developing countries: a critical review of available information. *Wld Hlth Statistics Quart No. 2;* pp.52-91.

Saarinen, U.M. and Siimes, M.A. (1977) — Developmental changes in serum iron, total iron binding capacity, and transferrin saturation in infancy. *J Pediatr* 91:875-877.

Salmi, T., Hannien, P. and Peltonen, T. (1963) — Applicability of chelated iron in the care of prematures. *Acta Pediatr Scand* 140 (Suppl):114-115.

Sbarra, A.J., Selvaraj, R.J., Paul, B.B., Strauss, R.R., Jacobs, A.A. and Mitchell, G.W. (1974) — Bactericidal activities of phagocytes in health and disease. *Am J Clin Nutr* 27:629-637.

Schlesinger, L. and Stekel, A. (1974) — Impaired cellular immunity in marasmic infants. *Am J Clin Nutr* 27:615-620.

Scott, J.M. and Weir, D.G. (1976) — Folate composition, synthesis and function in natural materials. *Clinics in Hematology* 5(3):547-568.

Seth, V. and Chandra, R.K. (1972) — Opsonic activity, phgocytosis and bactericidal capacity of polymorphs in undernutrition. *Arch Dis Child* 47:282-284.

Shaw, R. and Robertson, W.O. (1963) — Anemia among hospitilized infants. *Ohio Med J* 60:45. Cited in: Pearson and Robinson 1976.

Shott, R.J. and Andrews, B.F. (1972) — Iron status of a medical high-risk population at delivery. *Am J Dis Child* 124:369-371.

Siimes, M.A., Addiego, J.E. and Dallman, P.R. (1974) — Ferritin in serum : the diagnosis of iron deficiency and iron overload in infants and children. *Blood* 43:581-590.

Silber, R. and Moldow, C.F. (1970) — The biochemistry of B_{12} mediated reactions in man. *Am J Med* 48:549-554.

Singla, P.N., Chand, S., Khanna, S. and Agarwal, K.N. (1978) — Effect of maternal anemia on the placenta and the newborn infant. *Acta Pediatr Scand* 67: 645-648.

Sinhu, S.S. (1974) — Serum levels of iron, folic acid and vitamin B_{12} in the maternal and cord blood in the three major ethnic groups in Malaysia. *Indian Padiatr* 11:775-780. Cited in: Fairbanks, 1978.

Smith, E.L. (1965) — Vitamin B_{12}. Methuen & Co. Ltd: London.

Smythe, P.M., Schonland, M., Brereton-Stiles, G.G., Coovadia, H.M., Grace, H.J., Loening, W.E.K., Mafoyane, A. and Parent, M.A. (1971) — Tymolymphatic deficiency and depression of cell mediated immunity in protein-calorie malnutrition. *Lancet* 2:939-943.

Sood, S.K., Banerji, L. and Ramalingaswami, V. (1968) — Geographic pathology of iron deficiency with special reference to India. II. Quantitation of iron stores by repeated phlebotomy in Indian volunteers. *Am J Clin Nutr* 21: 1149-1155.

Sood, S.K., Ramachandran, K., Mathur, M., Gupta, K., Ramalingswamy, V., Swarnabai, C., Ponniah, J., Mathan, V.I. and Baker, S.J. (1975) — WHO sponsored collaborative studies on nutritional anemia in India. I. The effects of supplemental oral iron administration to pregnant women. *Quart J Med* 44:241-258.

Spiro, T. G. and Saltman, P. (1974) — Inorganic chemistry. In: Iron in Biochemistry and Medicine. Jacobs, A. and Worwood, M.(eds). Academic Press: London; pp. 1-28.

Sproule, B.J., Mitchell, J.H. and Miller, W.F. (1960) — Cardiopulmonary physiological responses to heavy exercise in patients with anemia. *J Clin Invest* 39:378-388.

Srikantia, S.G., Prasad, J.S., Bhaskaram, C. and Krishnamachari, K.A.V.R. (1976) — Anemia and immune response. *Lancet* 1:1307-1309.

Stenman, U. (1976) — Intrinsic factors and the vitamin B_{12} binding protiens. *Clinics in Hematology* 5:(3):473-495.

Stockstad, E.L.R. and Koch, J. (1967) — Folic acid metabolism. *Physiol Rev* 47:83-116.

Strauss, M.B. (1933) — Anemia of infancy from maternal iron deficiency in pregnancy. *J Clin Invest* 12:345. Cited in: Baker and DeMaeyer, 1979.

Strauss, R.G. (1978) — Iron deficiency, infections, and immune function:a reassessment. *Am J Clin Nutr* 31:660-666.

Sturgeon, P. (1959) — Studies of iron requirements in infants. III. Influence of supplemental iron during normal pregnancy on mother and infant. *Brit J Hematol* 5:45-55.

Sullivan, L.W. (1970) — Differential diagnosis and management of the patient with magaloblastic anemia. *Am J Med* 48:609-617.

Swendseid, M.E., Bird, O.D., Brown, R.A. and Bethell, F.H. (1947) — Metabolic function of pteroylglumtamic acid and its hexaglutamyl conjugate. II. Urinary excretion studies on normal persons: Effect of a conjugase inhibition. *J Lab Clin Med* 32:23-27.

Tamura, T. and Stokstad, E.L.R. (1973) — The availability of food folate in man. *Brit J Hematol* 25:513-532.

Tasker, P.W.G. (1951) — Anemia in the Indian labour force of some rubber estates in Malaya. *Med J Malaya* 6:10-14.

Tasker, P.W.G., Whelan, J.F.P and Cheek, E. (1952) — Further investigations on anemias of southern Indian rubber estate labourers. *Med J Malaya* 7:25-35.

Tasker, P.W.G. (1956) — Studies of the nutritional anemias of Malaya : the significance of the giant stab cells (as seen in the bone marrow of patients suffering from severe nutritional anemias). *Trans Royal Soc Trop Med Hyg* 50: 460-464.

Takser, P.W.G., Richardson, A.M. and Llewllyn-Jones, D. (1956) — Anemia in pregnancy as encountered in Malaya. *J Obstet Gynecol* 63:409-414.

Tasker, P.W.G. (1958a) — Studies of the nutritional anaemias in Malaya : the influence of hookworm infection. *Med J Malaya* 13(2):159-164.

Tasker, P.W.G. (1958b) — Anemia in pregnancy : a five-year appriasal. *Med J Malaya* 13:3-10.

Takser, P.W.G. (1961) — Blood loss from hookworm infection. *Trans Royal Soc Trop Med Hyg* 55:36-39.

Tee, E.S. (1982) — Nutrient composition of Malaysian foods : a preliminary table. *Report of the Division of Human Nutrition, Institute for Medical Research, Kuala Lumpur.*

Tee E.S., Kandiah, M., Jaafar Ali, Kandiah, V., Mohd. Rusli Zahari, Kuladevan, R. and Zulkafli Hamzah (1984) — Nutritional Anemia in pregnancy: a study at the Maternity Hospital, Kuala Lumpur. *Malaysian J Reprod Health* 2(1): 32-50.

Temperley, I.J., Meehan, M.J.M. and Gatenby, P.B.B. (1968) — Serum folic acid levels in pregnancy and their relationship to megaloblastic marrow change. *Brit J Hematol* 14:13-19.

Thanangkul, O. (1980) — Nutritional anemia with special reference to iron deficiency : relation to other nutritional deficiencies. In Nutrition and Food Science. Present Knowledge and Utilization. Volume 3. Nutritional Biochemistry and Pathology. Santos, W., Lopes, N., Barbosa, J.J., Chaves, D. and Valente, J.C. (eds). Plenum Press : N.Y.; pp. 203-218.

Thomas, W.J., Koening, H.M., Lightsey, A.L. and Green, R. (1977) — Free erythrocyte porphyrin : hemoglobin rations, serum ferritin, and transferrin saturation levels during treatment of infants with iron-deficiency anemia. *Blood* 49:455-462.

Toepfer, E.W., Zook, E.G., Orr, M.L. and Richardson, L.R. (1951) — Folic acid content of foods. *United States Department of Agriculture, Agricultural Handbook No. 29,* Washington, D. C.; Cited in: WHO, 1972.

Torrance, J.D. and Bothwell, T.H. (1980) - Tissue iron stores. In: Iron. Methods in Hematology, Volume 1. Cook, J.D. (ed). Churchill Livingstone: N.Y. ; pp. 90-115.

Viteri, F.E., Torun, B. (1974) — Anemia and physical work capacity. *Clinics in Hematology* 3(3):609-626.

Viteri, F.E., Alvarez, E., Bulux, J., Gonzales, H., Pineda, O., Mejia, L.A., Batres, R.B. and Torun, B (1981) — Iron fortification in developing countries. In: Nutrition in Health and Disease and International Development. Symposia from the XII International Congress of Nutrition. Harper, A.E. and Davis, G.K. (eds). Alan R. Liss Inc: N.Y.; pp. 345-354.

Voorhess, M.L., Stuart, M.J., Stockman, J.A. and Oski, F.A. (1975) — Iron deficiency anemia and increased urinary norepinephrine execretion. *J Pediatr* 86:542-547.

Wagner, A., Fortier, N., Giroux, A., Lukes, J. and Snyder, L.M. (1979) — Catecholamines in adult iron deficiency patients. *Experientia* 35:681-682.

Wadsworth, G.R. and Lee, T.S. (1959) — Hemoglobin levels of schoolchildren in rural health district of Malaya, and of pregnant women in Malaya and Singapore. *J Trop Pediatr* 5:67-72.

Walters, G.O., Miller, F.M. and Worwood, M. (1973) — Serum ferrition concentration and iron stores in normal subjects: *J Clin Pathol* 26:770-772.

Webb, T.E. and Oski, F.A. (1973) — Iron deficiency anemia and scholarstic achievement in young adolescents. *J Pediatr* 82:827-830.

Weinberg, E.D. (1978) — Iron and infection. *Microbiol Rev.* 42:45-66.

Whiteside, M.G., Ungar, B. and Cowling, D.C. (1968) — Iron, folic acid and vitamin B_{12} levels in normal pregnancy,and their influence on birthweigh and the duration of pregnancy. *Med J Aust* 1:338-342.

WHO, (1959) — Iron deficiency anemia : Report of a study Group. *Technical Report Series No. 182;* WHO: Geneva.

WHO, (1968) — Nutritional anemias : Report of WHO Scientific Group. *Technical Report Series No. 405;* WHO: Geneva.

WHO (1970) — Requirements of ascorbic acid, vitamin D, vitamin B_{12}, folate and iron. *Technical Report Series No.452;* WHO Geneva.

WHO (1971) — Food fortification and protein-calorie malnutrition. *Technical Report Series No. 477.* WHO: Geneva.

WHO (1972) — Nutritional anemias : Reports of WHO Group of Experts. *Technical Reports Series No. 503;* WHO: Geneva.

WHO (1975) — Control of nutritional anemia with special reference to iron deficiency. Report of an IAEA/USAID/WHO Joint Meeting. *Technical Report Series No. 580;* WHO: Geneva.

Willoughby, M.L.N. and Jewell, F.J. (1966) — Investigation of folic acid requirement in pregnancy. *Brit Med J* 2: 1568-1571.

Willoughby, M.L.N. (1967) — An investigation of folic acid requirement in pregnancy. II. *Brit J Hematol* 13:503-509.

Willoughby, M.L.N. and Jewell, F.G. (1968) — Folate status throughout pregnancy and in postpartum period. *Brit Med J* 4:356-360.

Woodruff, A.W. (1982) — Recent work concerning anemia in the tropics. *Seminars in Hematology* 19(2):141-147.

Worwood, M. (1980) — Serum ferritin. In: Iron. Methods in Hematology, Volume 1; Cook, J.D. (ed). Churchill Livingstone: N.Y.; pp. 59-89.

Worwood, M. (1982) — Ferritin in human tissues and serum. *Clinics in Hematology* 11:275-307.

Yusufji, D., Mathan, V.I. and Baker, S.J. (1973) — Iron, folate and vitamin B_{12} nutrition in pregnancy : a study of 1000 women from Southern India. *Bull Wld Hlth Org* 48:15-22.

Zalusky, R. and Herbert, V. (1961) — Megaloblastic anemia in scurvy with response to 50 microgram of folic acid daily. *New Engl J Med* 265:1033-1038.

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