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# **NUTRITIONAL ANAEMIAS**

**Report of a  
WHO Scientific Group**

**WORLD HEALTH ORGANIZATION**

GENEVA

1968

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## WHO SCIENTIFIC GROUP ON NUTRITIONAL ANAEMIAS

Geneva, 13-17 March 1967

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## NUTRITIONAL ANAEMIAS

### Report of a WHO Scientific Group

A WHO Scientific Group on Nutritional Anaemias was convened in Geneva from 13 to 17 March 1967.

The meeting was opened by Dr J. Karefa-Smart, Assistant Director-General, who welcomed the participants on behalf of the Director-General. Dr M. M. Wintrobe was elected Chairman, Dr D. L. Mollin Vice-Chairman, and Dr V. Herbert Rapporteur.

### INTRODUCTION

Nutritional anaemia is defined as a condition in which the haemoglobin 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. However, in defining a departure from normality, it must be recognized that normal haemoglobin levels vary with age, sex, weight, physiological status, and altitude. Anaemia is considered to be a late manifestation of nutritional deficiency, and even mild anaemia is not the earliest sign of such a deficiency. In this report, the terms iron deficiency and vitamin B<sub>12</sub> or folate deficiency are used in preference to iron deficiency anaemia and megaloblastic anaemia, respectively, since anaemias are a late manifestation of these deficiencies and the object of therapy is to correct the underlying deficiency rather than merely its manifestation.

Nutritional anaemias are an important nutritional problem affecting large population groups in most developing countries. Accordingly, the Joint FAO/WHO Expert Committee on Nutrition has recommended that WHO initiate and encourage the investigation of blood dyscrasias believed to be the result of malnutrition, with particular attention to the relative roles of dietary deficiencies and parasitism in the pathogenesis of nutritional anaemias. The Committee has also called attention to the problem of anaemias of pregnancy, which are considered to be of great public health importance, particularly in developing countries. In 1958, WHO convened a Study Group<sup>1</sup> to review the information available on iron deficiency anaemia. The Study Group recommended that investigations be under-

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<sup>1</sup> See *Wld Hlth Org. techn. Rep. Ser.*, 1959, No. 182.

taken of (a) the absorption of iron from foods in tropical countries, (b) dermal losses of iron in tropical regions, (c) the role of hookworm infection in anaemia, (d) tissue iron stores, and (e) the role of protein deficiency in anaemia in infants and children in the tropics. In 1961 WHO set up a collaborative study of some of these subjects.

It was soon recognized that very little was known about the prevalence of megaloblastic anaemias of nutritional origin. Although there was no conclusive evidence that they presented a public health problem, there was sufficient indication that the lack of such evidence was largely the result of the inadequacy or unsuitability of methods for prevalence studies. In 1961-62 WHO arranged a rapid survey of the seriousness of the problem of nutritional anaemias, particularly those characterized by megaloblastosis. Largely as a result of this survey WHO convened, with the financial support of the US National Institutes of Health, a meeting in 1962 to discuss the feasibility of developing methods for field application. It was concluded at this meeting that the nutritional anaemias of greatest importance were those attributable to deficiencies of iron, folate, and vitamin B<sub>12</sub>. Deficiencies of certain other nutrients (e.g., proteins) no doubt result in anaemia, but will probably be recognized only after iron, folate, and vitamin B<sub>12</sub> deficiencies have been excluded.

At the 1962 meeting, it was noted that some criteria (based on the levels of iron, folate, and vitamin B<sub>12</sub> in blood serum and on morphological changes in red and white blood cells as seen in peripheral blood) were available for detecting deficiencies. However, improved criteria were necessary, and their usefulness for detecting deficiency states that lead to anaemia required investigation. It was suggested that studies of the following characteristics of single specimens of blood and serum should be made: (a) whole blood: haemoglobin, packed cell volume, average lobe count of polymorphonuclear leucocytes, and morphological changes in red blood corpuscles; and (b) serum: iron, percentage saturation of transferrin, vitamin B<sub>12</sub> (assayed by *Euglena gracilis* or *Lactobacillus leichmannii*), and folate (assayed by *Lactobacillus casei*).

These measurements were considered to be the simplest and most useful of those for which high precision can be achieved.

As an outcome of the above recommendation, a collaborative study of anaemia in pregnancy was undertaken in 1963 in India, Israel, Mexico, Poland, South Africa, the United Kingdom, the USA, and Venezuela. Particular attention was to be given to the study of (1) tissue stores of iron; (2) the absorption of food iron; (3) dermal and total losses of body iron; and (4) the role of hookworm infection in anaemias associated with pregnancy. It was also suggested that the procedures for examining blood and serum recommended by the 1962 meeting be tested in the collaborative study. WHO established reference centres to standardize the methods to be used in the study, to ensure the cross-checking

of laboratories taking part, to assist investigators who lacked suitable facilities for the necessary procedures, and to train personnel.

The progress of the study was reviewed at a meeting held in Geneva in September 1963. Soon afterwards, WHO convened a meeting in Caracas, Venezuela, at which plans for studies of nutritional anaemia in Latin America and the Caribbean Region were outlined.

When the collaborative study had been in progress for a further 3 years, WHO convened the present Group to review the progress that had been made.<sup>1</sup> In addition to reviewing the over-all progress of the studies, the Group discussed relevant problems such as nutritional requirements of iron, folate, and vitamin B<sub>12</sub>.

## 1. THE PROBLEM

Many reports of investigations of nutritional anaemias have been published. Although most of these have been concerned with the situation in developing countries, similar studies have been undertaken in developed countries, where the results of several surveys have recently been published. There is good evidence that the prevalence of iron deficiency anaemia in children and women of child-bearing age is high in many areas of the world. However, there is a general lack of accurate data on its prevalence throughout the world. Most of the available information is incomplete, and in many investigations the population samples have been small and ill-defined or limited to special groups. True random samples of populations have rarely been surveyed; consequently, the prevalence figures that are available are of limited value. Methods used to measure different factors have been variable and often of poor quality. Furthermore, results have not always been comparable, since they have been expressed in different ways. These factors have added to the difficulty of obtaining reliable information on prevalence in different countries or regions.

To date, WHO-sponsored studies, using standard methods, indicate that the prevalence of anaemia in pregnant women in different parts of the world ranges from 21% to 80%. However, serum iron, vitamin B<sub>12</sub>, and folate levels indicate a much higher figure. For example, iron deficiency (as indicated by the percentage saturation of transferrin) was present in 40–99% of the pregnant women studied and was undoubtedly responsible for the major proportion of anaemia.

Less information is available on anaemias resulting from folate or vitamin B<sub>12</sub> deficiency. However, it is clear that such deficiencies occur in

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<sup>1</sup> The results of the studies undertaken in South Africa were largely unavailable to the Scientific Group, and consequently are not reviewed in this report.

substantial numbers and may at times be as important as iron deficiency as a cause of anaemia.

## 2. STANDARDIZATION OF METHODS

### Vitamin B<sub>12</sub> and folate in serum

The results of vitamin B<sub>12</sub> and folate assays made by collaborating laboratories have shown reasonably good agreement, owing to the use of standards provided by WHO. However, the degree of variation has been sufficiently great for it to be agreed that greater standardization of techniques is essential if the study is to continue. In this connexion, the Group make the following recommendations :

(1) A designated reference centre should make freeze-dried samples of sera available to the other reference centres and to certain collaborating laboratories, to enable these centres to prepare bulk serum standards. Sera containing low, near-normal, and normal amounts of folate and vitamin B<sub>12</sub> should be issued for this purpose.

(2) As a further control, samples of one known and one unknown freeze-dried serum should be issued to the same laboratories at intervals of 2-3 months.

(3) Large batches of crystalline folic acid and crystalline cyanocobalamin should be issued to the reference centres and collaborating laboratories.

(4) The recent introduction of the radioactive dilution method for measuring vitamin B<sub>12</sub> concentrations in serum and tissues makes it desirable that the results given by this technique be compared with those given by microbiological assay methods. The isotope method may make it possible to measure vitamin B<sub>12</sub> in liver specimens preserved in formol.

### Iron in serum

Collaborating laboratories have carried out a number of determinations of iron standards. The results have shown improvement as the experience of the laboratories has increased, but they are not yet satisfactory. It is recommended that in future studies each laboratory carry out a determination of an iron standard with each batch of serum determinations. In addition, a known serum standard should be tested once a week, and samples from the reference centres should be tested once a month. The samples for such standardizations should include sera of high, intermediate, and low iron level. It must be accepted that such cross-checking will always be necessary when a number of laboratories are involved in collaborative studies.

It is recommended that a reference centre be established for the determination of serum iron and iron binding capacity. Such a centre should establish procedures for checking the collaborating laboratories, and should keep in touch with the Expert Panel on Iron of the International Committee for Standardization in Haematology, which is concerned with the standardization of methods for determining serum iron and iron binding capacity.

### Haemoglobin

It is essential that haemoglobin determinations be standardized. The procedure<sup>1</sup> recommended by the International Committee for Standardization in Haematology should be followed, under the supervision of the designated iron reference centre.

### 3. CRITERIA FOR THE DIAGNOSIS OF ANAEMIA

In detecting and evaluating an anaemia problem in a community, reference standards are necessary, even though they may be somewhat arbitrary. The report<sup>2</sup> of the 1958 WHO Study Group recommended haemoglobin values below which anaemia could be considered to exist. These figures were chosen arbitrarily and it is still not possible to define normality precisely.<sup>3</sup> However, more recent data<sup>4</sup> indicate that the values given previously should be modified. It is recommended that, in future studies, anaemia should be considered to exist in those whose haemoglobin levels are lower than the figures given below (the values given are in g/100 ml of venous blood of persons residing at sea level):

children aged 6 months to 6 years :	11
children aged 6-14 years :	12
adult males :	13
adult females, nonpregnant :	12
adult females, pregnant :	11

At all ages the normal mean corpuscular haemoglobin concentration should be 34. Consequently, the haematocrit values corresponding to the haemoglobin concentrations given above may be obtained by multiplying

<sup>1</sup> International Committee for Standardization in Haematology (1967) *Brit. J. Haemat.*, 13 (Suppl.), 71.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1959, No. 182, p. 4.

<sup>3</sup> Wintrobe, M. M. (1967) *Clinical hematology*, 6th ed., Philadelphia, Pa., Lea & Febiger.

<sup>4</sup> Natvig, K. (1966) *Acta med. scand.*, 180, 613; Tibblin, G., unpublished observations; Kilpatrick, G. S. & Hardisty, R. M. (1961) *Brit. med. J.*, 1, 778; De Leeuw, N. K. M., Lowenstein, L. & Hsieh, Y. S. (1966) *Medicine (Baltimore)*, 45, 291; Sturgeon, P. (1959) *Brit. J. Haemat.*, 5, 31.

by 3. More than 95% of normal individuals are believed to show haemoglobin levels higher than the values given, which are appropriate for all geographic areas; however, the values must be modified for persons who reside at higher altitudes.

#### 4. WHO STUDIES OF ANAEMIAS OF PREGNANCY

During the past few years, WHO has sponsored or collaborated in a number of studies of nutritional anaemias of pregnancy in India, Israel, Mexico, Poland, and Venezuela. The results of these studies are given in tabular form in the Annex, and the principal findings are summarized in the following sections.

##### Israel

Since the end of 1963, a group of workers from the Hadassah University Hospital, Jerusalem, have studied all pregnant women in a rural community, in Upper Galilee. Most of these women are recent immigrants from North Africa and the Middle East. The results of the studies up to the end of 1965<sup>1</sup> indicated that more than 22% of women in the second and third trimester of pregnancy had haemoglobin levels below 10 g/100 ml and 35% had levels of 10.1–11 g/100 ml. Determinations of serum iron, vitamin B<sub>12</sub>, and folate, and of whole blood folate, indicated that anaemia was usually the result of iron deficiency, frequently combined with folate depletion (manifested by red cell folate levels of less than 100 ng/ml) and occasionally with a drop in serum vitamin B<sub>12</sub>. Since publication of the results of this study, the community has become economically depressed, and another survey has been made. The data presented in the Annex were obtained in the second survey, which covered 100 pregnant women, 100 nonpregnant women, and 66 males.

##### Warsaw, Poland

A survey of 220 women in the last trimester of pregnancy in Warsaw showed 21.8% of the women to have haemoglobin levels lower than 11.0 g/100 ml. There was almost no evidence of vitamin B<sub>12</sub> and folate deficiency. It was reported that pregnant women in Poland are usually well-nourished and often overweight, and that in large towns they are periodically examined as out-patients and, as a rule, treated with multi-vitamin and iron preparations.

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<sup>1</sup> See Rachmilewitz, M. et al. (1966) *Israel J. med. Sci.*, **2**, 472.

**New Delhi, India**

During the early stages of the present studies, a survey was made of the occurrence of anaemia in pregnant women in Delhi. Of a group of 1348 women, most of whom were in the second and third trimesters of pregnancy, 69% had a haemoglobin level below 11.0 g/100 ml, 38% had a transferrin saturation of less than 15%, and 35% had a serum vitamin B<sub>12</sub> level below 80 pg/ml.

Following this preliminary study, attention was given to rural populations, and a survey was made of 100 pregnant women (most of whom were in the second and third trimesters of pregnancy) and 95 nonpregnant women living in a typical rural community 30 miles south of Delhi. The data presented in the Annex were obtained in this study. It will be seen that 80% of the pregnant women had a haemoglobin level below 11.0 g/100 ml, 51.7% had a transferrin saturation below 15%, and 49% had a serum vitamin B<sub>12</sub> level below 80 pg/ml.

**Vellore, Madras State, India**

A survey was conducted of 1000 women receiving antenatal care at the General Hospital Clinic, Vellore. Of these women, 61% were from a very low income group, and all were in the last trimester of pregnancy. A group of 100 nonpregnant women of similar age and socio-economic background, and 99 husbands of women admitted for delivery, were studied as controls.

The mean haemoglobin level was found to be significantly lower among the pregnant women (10.2 g/100 ml) than in the female controls (12.3 g/100 ml). Haemoglobin levels of less than 12 g/100 ml were found in 35% of the nonpregnant women, and levels of less than 11 g/100 ml were found in 56% of the pregnant women. In 15% of the pregnant women, the levels were less than 8.0 g/100 ml. Iron deficiency, as indicated by low serum iron levels and decreased transferrin saturation, was found in 99% of the pregnant women.

Hookworm infection was present in 35% of the 542 women examined for this condition, and was associated with a mean reduction of 1.6 g/100 ml of haemoglobin (as compared with uninfected women).

Evidence of megaloblastic change was found in the bone marrow of 66% of the women at term, the change being severe in 28% of the women. Subnormal serum folate levels (less than 6 ng/ml) were found in 70% of the pregnant women; in 9% of the women, levels lower than 3.0 ng/ml were found. Subnormal vitamin B<sub>12</sub> levels (less than 140 pg/ml) were found in 49% of the pregnant women.

**Mexico**

A group of workers from the National Institute of Nutrition, Mexico City, carried out a survey in a rural community located 100 miles from

Mexico City, at an altitude of 2550 m. The group studied was composed of 128 women in the third trimester of pregnancy, 122 nonpregnant control female relatives of the pregnant women, and 114 control males. The families to which the subjects belonged were composed of an average of 5-6 persons and had an income of US\$ 1.00 per day.

At such high altitude the lower normal limits for haemoglobin are 13.3 g/100 ml for females and 14.7 g/100 ml for males (the figures given are the means minus 2 standard deviations), and for this reason it was decided to increase arbitrarily by 1.0 g/100 ml the levels used as criteria for detecting anaemia. On the basis of these modified criteria, the prevalence of anaemia was found to be 26.6% in pregnant women, 11.7% in nonpregnant women, and 0.9% in men. Transferrin saturation was lower than 15% in 61.2% of the pregnant women and in slightly less than one third of the nonpregnant women.

Subnormal serum folate levels (less than 6 ng/ml) were found in 60.5% of the pregnant women, 50% of the nonpregnant women, and 47.7% of the men, and the folate levels were lower than 3 ng/ml in 6.5%, 6.0%, and 3.5%, respectively, of the subjects in these groups. Subnormal serum vitamin B<sub>12</sub> levels (less than 140 pg/ml) were present in 15.3% of the pregnant women, and levels lower than 80 pg/ml were found in 7.1%.

#### **Caracas, Venezuela**

Workers in the PAHO Regional Reference Centre on Nutritional Anaemia at the Instituto Venezolano de Investigaciones Científicas, Caracas, studied 95 women in the last trimester of pregnancy, 107 nonpregnant women 1 year after the last delivery, and 53 men. All three groups were of a low socio-economic level. Haemoglobin levels of less than 11 g/100 ml were found in 37% of the pregnant women. There was evidence of iron deficiency, as indicated by low serum iron and decreased transferrin saturation, in approximately 60% of the pregnant women and 19% of the nonpregnant women. In approximately 15% of the pregnant women there was evidence of megaloblastic change in the bone marrow. Subnormal serum vitamin B<sub>12</sub> levels (less than 140 pg/ml) were found in 42.5% of the pregnant women, but in only 3% of the nonpregnant women; in 23% of the pregnant women, the level of serum vitamin B<sub>12</sub> was lower than 80 pg/ml. Serum folate values were lower than 6 ng/ml in 61.3% of the pregnant women, but such subnormal values were almost as common in the nonpregnant women and in the men.

#### **Therapeutic trials**

The cause of anaemia in pregnancy is best determined by carefully controlled therapeutic trials. The results of two such trials, in Madras, India, and in Israel, are available.

In Madras, a trial was conducted on 192 women, beginning in the first trimester of pregnancy, who were clinically normal and who had a haemoglobin level of 12 g/100 ml. The patients were divided into three approximately equal groups, each of which received 1 tablet daily containing 10 mg, 20 mg, and 50 mg, respectively, of iron (calculated as the element) in the form of ferrous fumarate. All the tablets also contained 5 mg of folic acid. In only 5 of the 192 subjects did the haemoglobin level fall below 10 g/100 ml.

In Israel, a therapeutic trial was carried out on 184 pregnant women, of whom 73% had a haemoglobin level of 10 g/100 ml, or less, at the beginning of the experiment. Each patient was given 1 capsule daily containing 100 mg of iron (calculated as the element), 5 mg of folic acid, and 100  $\mu$ g of vitamin B<sub>12</sub>. The average duration of treatment was 4.9 months, and the women were studied up to delivery. The prevalence of anaemia dropped from 73% before treatment to 15% after treatment. Most of the women who did not respond to therapy had discontinued medication because of gastrointestinal disturbances or other causes. A control group of 219 nonanaemic pregnant women, whose haemoglobin levels were above 10 g/100 ml at the outset, received placebo therapy. In this group, a reduction in haemoglobin level of at least 0.5 g/100 ml was found in 75% of women at delivery; in 55% of these, the reduction was at least 1 g/100 ml.

A therapeutic trial is now being conducted on pregnant women on a tea estate in South India. The patients are divided into 5 groups, who receive the following daily medication: (1) a placebo; (2) 50 mg of iron; (3) 50 mg of iron and 1  $\mu$ g of vitamin B<sub>12</sub>; (4) 50 mg of iron and 200  $\mu$ g of folic acid; and (5) 50 mg of iron, 200  $\mu$ g of folic acid, and 1  $\mu$ g of vitamin B<sub>12</sub>.

## 5. IRON DEFICIENCY

### Absorption of food iron

The amount of iron absorbed by the body depends on the total amount in the diet, its absorbability, and the regulation of its absorption by the body. Previous studies of absorption have raised the question as to whether the availability of the iron in certain common foods is low.

The Scientific Group studied the results of studies of the absorption of iron from various foods. Radioactive iron was incorporated into foods by biosynthesis, and the foods were fed to both normal and iron-deficient subjects. The degree of iron absorption was determined 2 weeks later by measuring the amount of radioactivity in the red cell mass. An essential part of the experiment was the administration of a second dose of radioactive iron (as a ferrous salt) on the morning following administration of

the food. Normal and iron-deficient subjects were differentiated on the basis of the degree of transferrin saturation and of absorption of ferrous iron: persons whose transferrin saturation was less than 18%, or whose absorption of a dose of 5 mg of iron (as ferrous salt) exceeded 25%, were considered iron-deficient. Examination of the data suggested that the degree of iron absorption is the more valid of the two criteria. The studies confirmed earlier findings that iron derived from plant sources is less well absorbed than that from animal sources, even in iron-deficient subjects. In iron-deficient subjects, the extent of iron absorption from staple foods, such as maize and wheat, was less than 10%, whereas iron was absorbed from meat and fish products at a level of 20%. These basic absorption levels were modified when the foods were mixed, although the data are too limited for any quantitative conclusions to be drawn.

There is an obvious need for further studies of the absorption of iron from biosynthetically tagged foods. However, the information that is available indicates that it is necessary to express dietary iron intake in terms of both the total iron intake and the absorbability of this iron. Iron in diets containing large amounts of animal protein may be expected to be absorbed to the extent of 15–20%, whereas the absorption of iron from diets that are predominantly composed of vegetable foods may be as low as 5–10%. Future appraisals of dietary iron should take this information into account.

Since the information that can be obtained by the measurement of radioactivity resulting from the consumption of tagged foods is limited, it is recommended that attempts be made to define chemical forms of iron in food. If this could be done, a more systematic understanding of the absorbability of iron might be achieved.

### Loss of body iron

Physiological losses of body iron in man occur in the gastrointestinal tract, urine, and sweat and by the exfoliation of skin. The daily extent of these losses can only be estimated approximately. In the gastrointestinal tract, iron is lost through the exfoliation of the mucosa (about 0.1–0.2 mg of iron per day) and through the loss of blood (allowing for the reabsorption of haemoglobin iron, about 0.2–0.3 mg of iron are lost in this way each day). Urinary losses of iron amount to less than 0.1 mg per day. Owing to inconsistencies in the results of isotope and chemical studies, it is not possible to give precise figures for losses in the sweat and through the exfoliation of skin; however, the information that is available indicates these to be less than 1 mg of iron per day.

The best information available on losses of iron from the body has been derived from long-term studies of  $^{55}\text{Fe}$  turnover in the red cell mass in normal males. This method has been used to study subjects in temperate

and tropical regions and under conditions of slight and extreme humidity. The studies indicate that iron is lost from the body at the rate of approximately 10% per year, regardless of environmental temperatures, or—if certain assumptions are made as to the total miscible pool of body iron—at the rate of about 0.6–1.0 mg/day in the adult male. Some subjects have been found to show iron losses of about 20% per year. The causes of such losses are still under examination.

There is evidence that iron losses may depend on the body iron content. For example, if the body has an overload of iron, losses may amount to 2 mg/day, whereas in iron-deficient persons they may be appreciably less than 1 mg/day. Although it is important that more precise studies of the nature and extent of physiological iron losses be carried out, there is information to indicate that such losses, being quite limited, are not of great importance in the pathogenesis of iron deficiency in males. Careful attention should be given to blood donors, since two 500-ml donations of blood a year would, assuming a haemoglobin content of 13 g/100 ml, be equivalent to an iron loss of 1.2 mg/day, more than twice the normal daily loss.

In females, severe menstrual blood loss is a major physiological cause of iron deficiency. The daily loss of iron through menstruation ranges from 0.1 to 4.0 mg/day, the average being 0.7 mg.<sup>1</sup>

#### **Tissue iron stores**

Under normal conditions, about a quarter of the iron content of the body is present in storage depots such as the liver, bone marrow, and spleen. From a functional standpoint, the iron stores are principally a reserve that can be drawn upon when the need arises. Determination of the level of stored iron has been found to be of major importance in determining the iron status of patients. Such a determination is most conveniently made by histological examination of bone marrow in order to judge the amount of stainable iron present in reticulum cells. Since iron levels in the liver apparently reflect total body stores, the determination of iron in liver specimens obtained post mortem is useful in the study of iron deficiency in different population groups. It was felt that a fair estimate of the iron nutrition in different regions of the world might be obtained if the amount of stored iron in liver samples obtained post mortem could be estimated and compared. Towards this end, WHO organized a study in which specimens of liver obtained in different areas of the world were analysed for total and non-haem iron concentrations. A histochemical assessment of haemosiderin in stained liver sections was also made.

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<sup>1</sup> Hallberg, L. (1966) *Acta obstet. gynec. scand.*, **45**, 25; Hallberg, L. (1964) *Näringsforskning*, p. 1.

*Quantitative estimation*

The estimation of total iron concentration in 1-g aliquots of liver samples presented no major problems, since reproducible results can be obtained by conventional wet or dry ashing procedures and the method of Bothwell et al.<sup>1</sup> By subtracting haemoglobin iron (estimated by converting the haem pigment to the pyridine haemochromogen) from the total iron concentration, it was possible to obtain a quantitative measure of iron stores in the liver.

Samples of liver taken at autopsy from 3578 subjects in 16 countries were analysed in this manner. The samples were obtained from subjects who died as the result of accidents and from patients dying in hospitals. Considerable variation was found in the quantity of stored iron in subjects from different parts of the world. In most countries, the concentrations were higher in males than in females. In an effort to identify the population groups that might be exposed most directly to the effects of iron deficiency, the percentage of individuals in each group with liver iron concentrations lower than 50 µg/g was determined. The lowest iron stores were found in male Indians living in New Delhi, of whom 30% had virtually none. Subjects from Nigeria and the USA showed the highest levels, and those from other countries fell between these two extremes. The results of this pilot study indicate that the approach holds promise for the future, but there are certain doubts regarding their interpretation. For example, striking differences were found in the results obtained for both males and females by different centres in the USA. Even more puzzling were the discrepancies in the results obtained by different hospitals in the same city; for example, results obtained by one hospital in London were lower than those obtained by two other London hospitals.

*Histochemical assessment of haemosiderin*

The haemosiderin content of the same liver samples was evaluated histochemically. Specimens that had been preserved in neutral buffered formol were stained, examined, and graded according to an arbitrary system. All specimens were graded by one observer who had no knowledge of their origin. It was found that, within a given geographical area, the haemosiderin content of livers obtained from persons who had died as the result of an accident often differed considerably from that of livers of patients who had died in hospital. For example, the average amount of haemosiderin in livers obtained from those dying of accidents in Venezuela was significantly lower than that in livers obtained from those dying in hospital in the same area. This finding must be borne in mind in comparing liver haemosiderin levels in different geographical areas.

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<sup>1</sup> Bothwell, T. H., Roos, N. & Lifschitz, M. L. (1964) *S. Afr. J. med. Sci.*, **29**, 21.

The results of the haemosiderin study confirm the early impression of the extreme paucity of iron reserves in livers obtained from Indians in Delhi and Vellore. Liver samples from Mexico City and from South African Indians also showed very little stainable iron. South African Bantus showed the highest iron reserves. The levels found in other countries (e.g., Czechoslovakia, Sweden, the United Kingdom, the USA, and Venezuela) fell in between these two extremes. The levels found in males were higher than those found in females, the difference being of about the same order of magnitude as is found by chemical estimation. It is of interest that a significant number of specimens from the United Kingdom and the USA, where iron stores are significantly higher than in India and Mexico, contained negligible stores of iron as measured by the presence of stainable iron.

The method used seems to provide a rough approximation of iron stores in population groups and to differentiate geographical areas that are known to have marked differences in the frequency of iron deficiency anaemia; however, the significance of the data requires further investigation. Interpretation of the results is complicated by the wide variations that were found in the liver samples obtained. For example, livers from Western Europe and the USA were obtained from subjects whose age was almost twice that of subjects in other areas. Differences in sampling methods may also complicate the interpretation of the data. A further factor that might be expected to influence the results is the cause of death, since certain terminal illnesses might modify the level of stored iron in the liver.

#### *Future tissue iron studies*

As previously noted, preliminary results indicate that tissue iron analysis is an important method of evaluating the iron status of populations in different geographical areas. However, the Group recommended that if further studies are undertaken they should be carried out more thoroughly, with attention to population sampling and to special studies of young age groups, and that the results given by this method should be thoroughly evaluated. The results that have been obtained should be analysed in detail, and the histological and chemical methods should be compared, as soon as possible. Studies of tissue iron stores are being planned in England and the USA and possibly in Sweden. It is hoped that these studies will be undertaken only after careful analysis of the results that have been obtained to date, and that they will be designed so as to give comparable results. It is evident that sampling must be carried out much more carefully in the future.

### Role of parasitic infection in iron loss<sup>1</sup>

Measurements made by isotope techniques have shown that the loss of blood resulting from hookworm infection varies from 2 ml to about 100 ml per day, depending on the degree of infection. In a study of 54 clinical cases with pure *Necator americanus* infection, a high correlation was found between faecal blood loss measured with <sup>51</sup>Cr and hookworm infection estimated either by the number of parasites recovered ( $r = 0.65 \pm 0.13$ ) or by oviposition expressed as grams of faeces ( $r = 0.73 \pm 0.07$ ) or as number of eggs per day ( $r = 0.76 \pm 0.08$ ). It was calculated that faecal blood loss is about 0.03 ml per worm per day and 2.1 ml per 1000 eggs per gram of faeces. In pure *Ancylostoma duodenale* infection, blood loss is greater (0.15–0.26 ml per worm per day) than in *Necator* infection and about 4.5 ml of blood are lost per 1000 eggs. These results indicate that an infection with 250 *Necator* worms or with 5000 eggs per gram of faeces will result in an intestinal haemoglobin iron loss of about 5 mg daily, of which only 70–80% will be eliminated in the faeces. Consequently, infections with more than 5000 eggs will undoubtedly cause an imbalance in iron metabolism and induce, in the long run, iron-deficiency anaemia. A lesser degree of infection could also induce anaemia in populations whose food contains relatively low amounts of absorbable iron. Infection with 100 ancylostome worms will be sufficient to induce anaemia under comparable conditions.

*Trichuris trichiura* is another parasite that sucks blood from the intestinal mucosa. A preliminary study of 9 children with pure *Trichuris trichiura* infection showed them to be losing about 0.005 ml of blood per worm per day, about 0.25 ml per 1000 eggs per gram of faeces per day, and 0.8 ml per million eggs per day. It was estimated that infection with over 800 worms—i.e., more than 15 000 eggs per gram of faeces—would induce anaemia in children. Since heavy loads of this parasite are rarely found in adults, it is probably not one of the main causes of iron-deficiency anaemia in adults.

Chronic blood loss resulting in iron deficiency can also be caused by schistosome infections. The preliminary results of recent studies at the US Naval Medical Research Unit, Cairo, UAR, indicate that appreciable blood losses, resulting in the loss of 3–5 mg of iron per day, can result from infections with *S. haematobium* and *S. mansoni*. This finding, if confirmed, will be of considerable significance in regions where schistosomiasis is a major problem.

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<sup>1</sup> Recent publications on this subject include the following: Roche, M. & Layrisse, M. (1966) *Amer. J. trop. Med. Hyg.*, **15**, 1032; Martinez-Torres, C., Ojeda, A., Roche, M. & Layrisse, M. (1967) *Trans. roy. Soc. trop. Med. Hyg.*, **61**, 373; and Layrisse, M., Aparcedo, L., Martinez-Torres, C. & Roche, M. (1967) *Amer. J. trop. Med. Hyg.*, **16**, 613.

### Iron requirements

The optimum dietary requirements of iron may be defined as the amount and kind of food iron which, when absorbed, cover the physiological losses and demands of iron in all subjects under all physiological conditions, including growth and pregnancy.

The estimated daily iron requirements of children and adults are listed in Table 1. The figures in this table show that there is a wide variation in

TABLE 1. ESTIMATED DAILY IRON REQUIREMENTS \*

Group	Basic daily loss (mg)	Daily loss through menstruation (mg)	Daily requirement for growth or pregnancy (mg)	Total daily requirement (mg)
Children 1-4 years	0.15		0.25	0.4
Children 5-8 years	0.2		0.2	0.4
Children 9-12 years	0.3		0.5	0.8
Boys 13-16 years	0.5		0.6-1 +	1.1-1.5 +
Girls 13-16 years	0.5	0.1-2.6	0.6-1 +	1.2-4.1 +
Adult women of reproductive age	0.6	0.1-3.4		0.7-4.0
Pregnant women <sup>a</sup>				
1st and 2nd trimesters	0.6		0.1	0.7
3rd trimester	0.6		6.0	6.6
Adult nonmenstruating women	0.6			0.6
Adult men	0.6			0.6

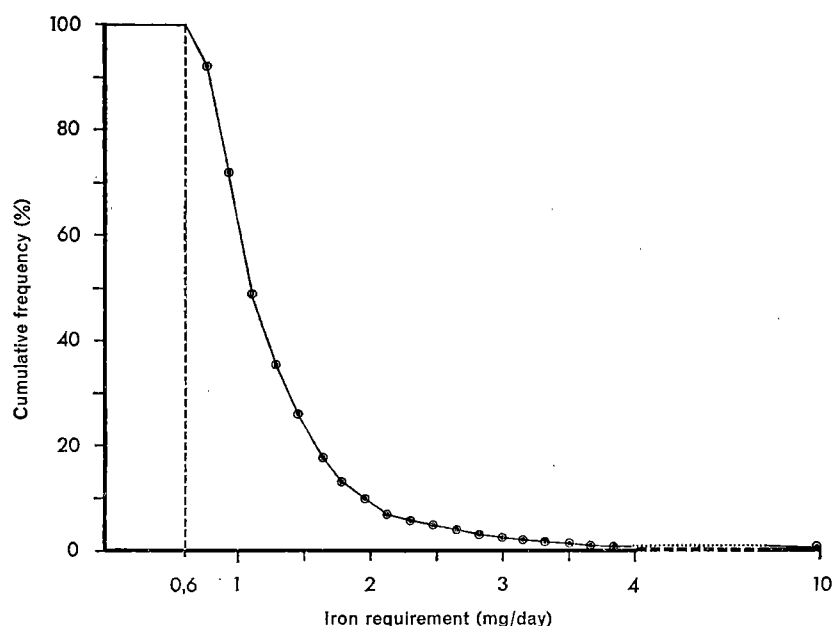
\* Data from Hallberg, L. (1964) *Näringsforskning*, 9, 1.

<sup>a</sup> The figures given are averages for the different trimesters. The extra iron requirement necessitated by pregnancy actually rises from zero at the 20th week to almost 8 mg/day at the 40th week of pregnancy (Hallberg, L. [1966], *Obstetrik & Gynekologi*, 3, 59). The average extra iron requirement during pregnancy is 2.4 mg/day; however, this average is highly artificial, since the requirement is so unevenly distributed throughout the three trimesters.

menstrual blood losses, the total daily requirement of iron by adult menstruating women ranging from 0.7 to 4.0 mg. This variation is made more apparent in the accompanying distribution curve.

Pregnant women need iron to cover their basic losses (0.6 mg per day for 300 days = 180 mg), the demands of the foetus (250-300 mg) and of the placenta (75 mg). In addition, 300-400 mg of iron are required to permit an increase of the haemoglobin mass. Consequently, the total iron demand during pregnancy amounts to about 900 mg. From the point of view of long-term balance, the iron required to increase the haemoglobin mass (400 mg) is not to be considered a loss. However, 200 mg are lost during and immediately after delivery, bringing the total iron requirement during pregnancy to about 700 mg.

## DISTRIBUTION CURVE OF IRON REQUIREMENT OF WOMEN OF REPRODUCTIVE AGE\*



\* Reproduced, with permission, from Hallberg, L. (1964) *Näringsforskning*, 9, 1.

Data presented during the meeting clearly showed that the iron in different foodstuffs varies considerably in absorbability. Consequently, in evaluating the adequacy of a given diet, it is necessary to know both its total iron content and the iron content of each different foodstuff. It is also necessary to know whether any of the foodstuffs will adversely affect the absorption of iron from other foodstuffs. Figures presented to the Group indicate that the absorption of iron from a given foodstuff by iron-deficient subjects may vary from less than 10% to slightly more than 20%, depending on the proportions of plant and animal foods in the diet (see p. 14). For example, it is estimated that the average absorption of iron is about 12% from a diet in which one third of the calories are supplied by meat.

Knowledge of the iron and calorie intake of different age and population groups is inadequate. There is also insufficient information on (a) the amount of iron in different foods, (b) the way in which food preparation may affect the absorption of iron, and (c) the amount of iron derived from cooking utensils and the absorbability of such iron. It seems, therefore, that further studies are necessary in order to obtain sufficient data for estimating nutritional iron requirements. The Group was informed that consideration is being given to the convening of a Joint FAO/WHO Expert

Committee for the purpose of establishing internationally acceptable human requirements of iron.

The most vulnerable group are women of reproductive age. If the dietary iron is to meet the demand in all menstruating women (i.e., the estimated maximum requirement of 4 mg per day), the diet should contain at least 30–40 mg of well absorbable iron per day (more if the iron is poorly absorbable). If the diet is to meet the demand in only 90% of the women, this figure may be reduced by 50%.

In estimating the dietary iron requirements of pregnant women, reliance should not be placed on the iron stores, since these have been found to be small or absent in most women. However, the iron requirement increases greatly during pregnancy, and the extremely high daily requirement during the last trimester cannot possibly be met from the food consumed during pregnancy. For this reason, the diet must be supplemented by the administration of medicinal iron, at least during the second half of pregnancy (see p. 25). The administration of supplementary iron is also recommended in areas where widespread intestinal parasitism (e.g., with hookworms) results in varying degrees of blood loss (see p. 25).

## 6. VITAMIN B<sub>12</sub> AND FOLATE DEFICIENCY

The results of the WHO studies to date indicate that folic acid deficiency is common, and vitamin B<sub>12</sub> deficiency relatively rare, in pregnancy. However, although these results are, in general, representative of the communities investigated, they do not accurately reflect the occurrence of deficiency throughout the world.

### Criteria for the diagnosis of deficiency

The accuracy of morphological diagnoses of vitamin B<sub>12</sub> and folate deficiencies varies with the experience and skill of the observer, and the results are difficult to standardize. For this reason, the Group recommended that, in any statistical evaluation, peripheral blood and bone marrow films should be examined at only one centre. They also pointed out that the only way to determine the extent to which vitamin B<sub>12</sub> or folate deficiency contributes to anaemia is clinical and haematological examination before and after treatment.

Deficiency in vitamin B<sub>12</sub> and folate may be diagnosed by determining the serum levels of these substances. The results are interpreted as shown in Tables 2 and 3.

The red cell folate level gives a more accurate indication of the state of tissue stores than does the serum folate level. However, the estimation of red cell levels necessitates the collection of a separate specimen, which

TABLE 2. SERUM VITAMIN B<sub>12</sub> LEVELS

Level (pg/ml)	Interpretation
200-960	normal range
140-200	diagnostically indeterminate
80-140	suggestive of deficiency
< 80	deficiency

TABLE 3. SERUM FOLATE LEVELS

Level (ng/ml)	Interpretation
> 20	elevated
6-20	normal range
3-5.9	possible deficiency
< 3	deficiency

may be a disadvantage in the field. Red cell folate levels may be low in patients with vitamin B<sub>12</sub> deficiency anaemia, but the significance of this fact is uncertain. The results of red cell folate determinations vary, depending on the method used. It is recommended that the whole blood folate level be determined and the result corrected for the haematocrit and the serum folate concentration. With this method, a level of less than 100 ng/ml indicates deficiency.

### Requirements <sup>1</sup>

#### Vitamin B<sub>12</sub>

The estimated minimum adult daily requirement of vitamin B<sub>12</sub> is about 0.1-0.3 µg (available to the tissues). In pregnancy and hypermetabolic states, the daily requirement may be greater (perhaps as great as 1 µg). The absorbability of vitamin B<sub>12</sub> in food is not well understood, and information on this subject is urgently needed.

<sup>1</sup> For further information, see: Herbert, V. (1966) *Nutritional requirements for vitamin B<sub>12</sub> and folic acid*. In: *Proceedings of the Eleventh Congress of the International Society of Haematology*, Sydney, N.S.W., Australia, Blight.

*Folate*

The estimated minimum adult daily requirement of folate is 50–100  $\mu\text{g}$  as pteroylglutamic acid (PGA). In pregnancy and in hypermetabolic states, requirements are increased. The amount of PGA required for prophylaxis in pregnancy is approximately 300  $\mu\text{g}/\text{day}$ . Adequate information is lacking on the absorbability of food folate and on the forms of folate in different foods and thorough research should be undertaken in these areas.

*Future studies*

Studies of vitamin B<sub>12</sub> and folate levels carried out with WHO support have been limited to pregnant women. It is recommended that further studies be carried out on nonpregnant women, men, children, and infants living in the communities in which pregnant women have already been studied.

## 7. PREVENTION OF NUTRITIONAL ANAEMIAS

In discussing methods for the prevention of nutritional anaemias, the Group gave particular attention to the prevention of iron deficiency.

### **General sanitary and dietary measures**

In areas where hookworm infection is the principal cause of iron deficiency anaemia, the best preventive measure is obviously the eradication of the parasite. However, experience has shown that this is not an easy task; it takes a long time and cannot be expected to give spectacular results. Such a sanitation programme must usually be closely linked to the raising of the standard of living of the population. Nevertheless, the Group is of the opinion that sanitary measures should be strengthened, particularly in rural areas where hookworm infection is prevalent.

In some countries, a decrease in the consumption of iron has resulted from the substitution of an iron-poor food for an iron-rich one, or from the introduction of more refined food processing methods. For example, the consumption of unrefined sugar (which contains a significant amount of iron) has decreased, and the consumption of refined sugar (which contains less iron) has increased. A similar trend has been observed even in some developed countries.

Education programmes, particularly for mothers (through maternal and child health centres) and for schoolchildren, should also be strengthened. Such programmes should encourage the eating of vegetables, pulses, and other foods that are rich in iron and discourage unsatisfactory cooking practices.

### Food enrichment

Long-term programmes—such as sanitation, education, and food production—must be supplemented by more immediate measures, such as the fortification of foods with iron, which is an urgent need in most countries of the world. The Group is of the opinion that such enrichment is potentially effective for preventing iron deficiency. The iron content of wheat flour and baby foods is now enriched in some countries, but little information is available on the effectiveness of this measure. Several problems are involved in the successful application of food enrichment. Dietary habits and food consumption vary in different parts of the world, necessitating careful studies to indicate the most appropriate food to be enriched and the most suitable iron compound to be used for enrichment. The Group believe the following steps to be necessary in developing an effective iron enrichment programme :

- (1) Collection of information on, and evaluation of the results of, programmes already in operation, covering (a) the foods that are enriched ; (b) the iron salts used for enrichment, and the amounts of such salts used ; and (c) the availability to the body of such iron.
- (2) The selection of foodstuffs that are most suitable for enrichment, taking into consideration the likelihood of their use by a given population.
- (3) The selection of a suitable iron compound as an enriching agent. This will involve studies of the absorbability of the iron from the food that is enriched, and of the stability of the enriched product. The level of enrichment will depend on the requirements of the body in different physiological conditions and on the absorbability of the iron.
- (4) A study of the technological processes to be used for enrichment and of the cost of producing and marketing the enriched product. The acceptability of the product should also be studied.
- (5) Pilot trials to determine the effectiveness of iron enrichment in different regions.

The series of steps outlined above is essential for the formulation of an iron-enrichment programme that may be confidently recommended for adoption by the Member States of WHO. Since iron deficiency anaemia is so widespread, the Group recommend that WHO consider the possibility of undertaking such studies at an early date.

It would also be advisable to consider the use of protein-enriched foods for preventing protein malnutrition in infants and children. In developing a protein-enrichment programme, the steps outlined above should be followed.

### **Iron supplementation in pregnancy**

Iron deficiency is widespread in pregnant women throughout the world. From considerations of iron balance it is clear that the requirements of pregnant women cannot be met during pregnancy by diet alone. Until the body iron stores of a given population are built up, the iron requirements of its pregnant women must be met by supplementation. Carefully controlled study to establish the optimum dose of iron is a matter of the greatest urgency. It is recommended that a study be carried out on 3 groups of pregnant women, to whom iron should be administered in the amounts of 20, 60, and 120 mg/day. A fourth group should receive no supplementary iron. The iron tablets used in the study should be specially prepared, and the extent of iron absorption should be determined by isotope techniques. Folate and vitamin B<sub>12</sub> should be administered to all the women, to exclude the possibility of complicating deficiencies with these substances.

Until the results of such a study are available, it is recommended that a dose of a ferrous salt containing at least 60 mg of elemental iron be given once daily during the second and third trimesters of pregnancy and during the first 6 months of lactation. This recommendation is consistent with that of the 1958 WHO Study Group on Iron Deficiency Anaemia.<sup>1</sup> However, the best long-term approach to the prevention of anaemia in pregnancy is fortification of the iron content of food.

### **Mass treatment of iron deficiency in schoolchildren**

Several studies have shown that good results can be obtained by the daily administration of small amounts of iron to schoolchildren, even those who are heavily infected with parasites that cause blood loss. However, the haemoglobin of such children returns to its pretreatment levels several months after treatment is discontinued.

The Group considered that in areas where iron deficiency anaemia is highly prevalent among schoolchildren, it would be useful to screen such children and treat those who are found to be suffering from the condition. Screening could be carried out by periodic surveys of parasitic infection and haemoglobin levels, which could be performed by auxiliary laboratory personnel under medical supervision. Combined deworming and administration of iron may give good results in an emergency. However, it is strongly recommended that before such a programme is undertaken at national level, appropriate trials be carried out in a pilot area to evaluate its effectiveness and cost.

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<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1959, No. 182, p. 12.

## 8. CO-ORDINATION OF RESEARCH ON NUTRITIONAL ANAEMIA

Organized research on nutritional anaemias has been greatly expanded in recent years. For several years before WHO established the present collaborative study in 1961, the Wellcome Trust of London had been sponsoring research on sprue, megaloblastic anaemia, and iron deficiency anaemia in certain tropical countries. Since 1961, other bodies have organized studies of anaemias. The Williams Waterman Fund has assisted in the establishment of a training and reference centre for anaemia studies in Caracas, Venezuela. The US National Institutes of Health, in association with PAHO, have sponsored investigations of nutritional anaemias in Latin America and the Caribbean. Similar studies have recently been undertaken in South-East Asia by the US-Japan Co-operative Medical Science Programme. In the United Kingdom, the Ministry of Health is carrying out a study of the nature of the folate deficiency problem in that country.

The general nature of the information sought in such studies, and the methods used, will probably be more or less similar. For this reason, it appears desirable to achieve co-ordination between the different groups undertaking such studies, so as to (a) avoid duplication of effort, (b) make the best use of available resources, (c) ensure the comparability of methods and results, and (d) pool the information that is obtained.

Co-ordination between different organizations must be flexible, and must be achieved largely through personal contact. For this reason, the Group feel it inadvisable to suggest any set way of achieving co-ordination. However, they recommend that WHO take the initiative in arranging, with other organizations, the frequent exchange of information on the progress of studies of nutritional anaemias. Such information should be periodically circulated to the participants in WHO collaborative studies.

### WHO collaborative studies

Although the WHO collaborative study undertaken in 1963 has yielded important information on nutritional anaemia, much remains to be done, and the Group makes the following suggestions for future studies.

Considerable progress has been made in the development of methods, but the procedures used are not foolproof and necessitate repeated checking and the maintenance of reliable standards. The establishment of reference laboratories has proved to be useful. However, continuous supervision and co-ordination are necessary if data from the different areas under study are to be dependable and interpretable. Such co-ordination could best be achieved by assigning the responsibility for all reference laboratories to a single individual.

Prevalence studies and therapeutic trials should be continued, and the data that have already been collected should be further analysed. Studies should be undertaken to determine the significance of relatively low levels of serum folate and vitamin B<sub>12</sub> as indices of impending or actual morbidity. Particular attention should be given to evaluation of the effectiveness of preventive measures. However, large-scale prophylaxis should not be undertaken before pilot studies have been carried out to test the effectiveness of the proposed measures under the conditions under which they are to be used.

Effective prophylaxis cannot be expected without an understanding of etiology and pathogenesis. Consequently, epidemiological and other studies of these factors should be undertaken at the same time as the prevalence studies and therapeutic trials.

In a large co-operative study, waste of time and effort can be minimized if the investigators meet periodically to discuss the progress of the study.

## 9. SUMMARY OF RECOMMENDATIONS

Further studies of the following topics should be undertaken :

- (1) The prevalence of iron, folate, and vitamin B<sub>12</sub> deficiency in infants and children of pre-school age, and the requirements of these nutrients by such children.
- (2) The absorption of food iron and the enrichment of the iron content of foods.
- (3) The quantity, nature, and absorbability of vitamin B<sub>12</sub> and folates in food, with attention to the effects of cooking, storage, and processing.
- (4) The pathogenesis of iron, folate, and vitamin B<sub>12</sub> deficiencies, with particular attention to the prevalence of malabsorption, especially tropical sprue.
- (5) The immunological diagnosis and prevention of parasitic diseases.
- (6) The best methods of eliminating deficiency states. Such therapeutic studies should be carefully planned, statistically controlled, and co-ordinated with those undertaken by other groups, and the absorbability of the therapeutic agents used should be checked periodically.

## ACKNOWLEDGEMENTS

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**Annex**

**RESULTS OF WHO STUDIES OF ANAEMIA  
OF PREGNANCY**

Some of the results of WHO studies of anaemia of pregnancy undertaken during the past few years in India, Israel, Mexico, Poland, and Venezuela are presented in tabular form on the following pages.

TABLE 1. AVERAGE VALUES AND STANDARD DEVIATIONS

Country	Subjects	Total number of cases	Haemoglobin (g/100 ml)		Packed cell volume (%)		Serum iron (µg/100 ml)		Total serum binding capacity (µg/100 ml)		Transferrin saturation (%)		Vitamin B <sub>12</sub> (pg/ml)		Serum folate, assayed by L. casei (ng/ml)	
			Av.	S. D.	Av.	S. D.	Av.	S. D.	Av.	S. D.	Av.	S. D.	Av.	S. D.	Av.	S. D.
Israel	Women : Pregnant	50-100	10.9	1.6	34.6	3.5	60.0	28.9	350	88.3	20.7	13.6	246	134.3	6.2	2.7
	Nonpregnant	97-100	12.5	1.1	41.2	3.1	86.5	36.8	237	74.8	42.5	28.1	498	215.6	7.8	4.5
	Men	54-66	14.2	1.3	44.8	3.2	81.0	31.3	207	40.2	41.2	19.8	479	124.0	10.1	5.0
Poland	Women, pregnant	210-220	12.1	1.3	36.9	3.4	73.3	38.0	335	92.3	19.9	10.8	263	111.3	14.2	9.6
India (Delhi)	Women : Pregnant	93-100	9.8	1.6	32.7	4.3	72.0	28.3	466	67.5	16.2	6.3	97	53.5		
	Nonpregnant	89-95	10.5	2.1	34.7	5.9	70.5	23.1	371	83.1	21.7	10.3	122	67.5		
India (Vellore)	Women : Pregnant	100	10.2	2.3	31.0	6.5	27.0	8.7	445	45.1	6.2	2.5	148	51.5	5.1	2.0
	Nonpregnant	99-100	12.3	1.9	37.9	5.6	57.4	43.2	308	68.7	13.6	11.2	215	109.9	9.3	4.8
Mexico	Men	99	15.0	1.7	45.2	4.4	100.0	25.3	266	48.7	39.1	12.0	197	87.3	8.1	3.5
	Women : Pregnant	85-128	12.9	3.1	39.2	5.2	74.1	36.4	518	81.4	15.2	8.6	292	130.9	6.2	4.6
Venezuela	Nonpregnant	110-122	14.3	1.8	44.1	6.0	87.9	44.3	380	69.9	24.4	13.2	426	139.5	9.1	12.9
	Men	93-114	16.4	1.1	49.9	3.2	111.9	45.9	326	47.2	35.4	13.3	370	107.6	9.0	8.2
	Women : Pregnant	62-95	11.3	1.3	36.8	5.0	47.1	48.3	513	93.6	14.8	9.1	188	143.3	5.4	2.6
	Nonpregnant	100-107	12.9	1.5	41.9	3.1	87.6	35.0	373	55.2	24.8	11.7	492	262.0	7.4	7.4
	Men	48-53	15.1	1.1	47.4	4.6	112.5	43.3	355	44.0	34.7	8.6	435	198.7	5.7	2.8

TABLE 2. HAEMOGLOBIN LEVELS

Country	Subjects	Total number of cases	Proportion (%) of cases with the following haemoglobin levels (mg/100 ml)										Proportion of cases with anaemia <sup>a</sup> (%)			
			<6	6-6.9	7-7.9	8-8.9	9-9.9	10-10.9	11-11.9	12-12.9	13-13.9	14-14.9		15-15.9	16-16.9	≥17
Israel	Women : Pregnant	100			2.0	14.0	21.0	10.0	25.0	19.0	9.0					47.0
	Nonpregnant Men	66					3.0 1.5	9.0	17.0	38.0 12.1	23.0 30.3	9.0 27.3	1.0 21.2	6.1	1.5	29.0 13.6
Poland	Women, pregnant	220				0.5	2.7	18.6	20.0	35.0	18.6	3.6	0.5	0.5		21.8
India (Delhi)	Women : Pregnant	100	1.0	4.0	12.0	16.0	14.0	33.0	7.0	12.0		1.0				80.0
	Nonpregnant	95	3.2	5.3	8.4	4.2	4.2	27.4	11.6	29.5	4.2	1.1	1.1			64.3
India (Vellore)	Women : Pregnant	100	10.0	2.0	3.0	7.0	13.0	21.0	24.0	12.0	6.0	2.0				56.0
	Nonpregnant Men	99			1.0	5.0	2.0	11.0	16.0	32.0	23.0	9.0	33.3	14.1	1.0 9.1	35.0 6.0
Mexico	Women : Pregnant	124		0.9	0.8		4.8	6.5	14.5	31.5	23.4	14.5	2.4		1.6	26.6
	Nonpregnant Men	110 111			0.9		1.8	1.8	2.7	3.6	21.8 0.9	25.5 6.3	26.4 23.4	12.7 41.4	1.8 27.9	11.7 0.9
Venezuela	Women : Pregnant	95			1.1	1.1	11.6	23.2	23.2	32.6	6.3	11.2	1.9	1.1	0.9	37.0
	Nonpregnant Men	107 52			0.9	0.9	1.9	0.9	10.3	33.6 1.9	37.4 11.5	36.5	19.2	23.1	7.7	14.9 1.9

<sup>a</sup> With the exception of Mexico (for which they are increased by 1 g/100 ml to correct for the high altitude), the haemoglobin levels considered to be diagnostic of anaemia are those recommended on p. 9.

TABLE 3. SERUM IRON

Country	Subjects	Total number of cases	Proportion (%) of cases with the following serum iron levels (µg/100 ml)										Proportion of cases with serum iron < 50 µg/100 ml	
			< 20	20-39	40-59	60-79	80-99	100-119	120-139	140-159	160-179	180-199		≥ 200
Israel	Women : Pregnant	100	7.0	16.0	31.0	17.0	17.0	9.0	1.0	2.0				36.0
	Nonpregnant Men	100 66	2.0 7.6	4.0 1.5	21.0 13.6	20.0 19.7	17.0 21.2	16.0 27.3	14.0 9.1	2.0	1.0	3.0		15.0 12.1
Poland	Women, pregnant	220	5.5	15.0	21.4	16.4	15.9	11.4	8.2	5.0	0.9		0.5	31.4
India (Delhi)	Women : Pregnant	100	3.0	5.0	16.0	42.0	25.0	2.0	3.0	2.0	2.0			10.0
	Nonpregnant	95	4.2	5.3	8.4	46.3	28.4	6.3	1.1					9.5
India (Vellore)	Women : Pregnant	100	16.0	76.0	7.0	1.0	11.0	8.0					1.0	99.0
	Nonpregnant Men	100 99	2.0 5.1	24.0 5.1	40.0 3.0	14.0 7.1	25.3	38.4	20.2	1.0				51.0 7.1
Mexico	Women : Pregnant	128	1.6	14.8	23.5	18.0	18.8	11.7	7.0	3.1	0.8		0.8	30.5
	Nonpregnant Men	122 114	3.3 2.6	9.8 1.8	16.4 6.2	16.4 12.3	13.1 11.4	16.4 20.2	13.9 21.1	4.9 13.2	3.3 7.9	1.6 0.9	0.8 2.6	22.1 6.2
Venezuela	Women : Pregnant	95	35.8	12.6	16.8	13.7	6.3	4.2	8.4				2.1	56.8
	Nonpregnant Men	107 53	0.9 7.5	4.7	13.1	26.2	22.4	16.8	5.6	5.6	2.8	1.9	3.8	14.0 7.5

TABLE 4. TOTAL SERUM BINDING CAPACITY

Country	Subjects	Total number of cases	Proportion (%) of cases with the following total serum binding capacities (ug/100 ml)												
			< 149	150-199	200-249	250-299	300-349	350-399	400-449	450-499	500-549	550-599	600-649	650-699	≥ 700
Israel	Women : Pregnant	80		2.5	13.8	16.3	16.3	10.0	25.0	15.0	1.3				
	Nonpregnant	97	10.3	25.8	18.6	19.6	19.6	5.2	1.0						
	Men	57	3.5	43.9	42.1	8.8	1.8								
Poland	Women, pregnant	220		1.8	1.8	9.1	25.9	25.0	13.2	10.9	6.4	2.7	2.3	0.9	
India (Delhi)	Women : Pregnant	93		1.1	1.1		2.2	6.5	26.9	25.8	30.1	6.5			
	Nonpregnant	89	1.1	3.4	4.5	7.9	21.3	22.5	19.1	19.1	1.1				
India (Vellore)	Women : Pregnant	100				2.0	3.0	4.0	45.0	41.0	5.0				
	Nonpregnant	99		2.0	13.1	35.4	31.3	10.1	6.1	1.0				1.0	
	Men	99			47.5	38.4	8.1	2.0	3.0		1.0				
Mexico	Women : Pregnant	126		0.8	0.8	9.1	23.1	4.8	17.5	19.0	23.8	22.2	9.5	1.6	1.6
	Nonpregnant	121			6.3	21.4	40.2	32.2	19.0	7.4	5.8	1.7			
	Men	112						26.8	5.4						
Venezuela	Women : Pregnant	62				1.6	1.6	8.1	17.7	14.5	21.0	17.7	9.7	8.1	
	Nonpregnant	106			1.9	7.5	23.6	34.0	25.5	7.5					
	Men	49			6.1	4.1	26.5	55.1	8.2						

TABLE 5. TRANSFERRIN SATURATION

Country	Subjects	Total number of cases	Proportion (%) of cases with the following transferrin saturation values (%)													
			0-10	10.1-15	15.1-20	20.1-30	30.1-40	40.1-50	50.1-60	60.1-70	70.1-80	80.1-90	90.1-100	100	< 15	< 18
Israel	Women : Pregnant	80	22.5	23.8	13.8	17.5	12.5	7.5	1.3	1.3	6.2	2.1	3.1	5.2	46.3	51.3
	Nonpregnant	97	6.2	5.2	8.3	20.6	19.6	13.4	6.2	5.2	3.5	1.8			11.4	16.6
	Men	57	7.0	1.8	3.5	15.8	24.6	7.0	26.3	7.0					8.8	8.8
Poland	Women, pregnant	220	18.6	21.4	14.5	27.3	13.6	3.6	0.9						40.0	50.9
India (Delhi)	Women : Pregnant	93	10.8	40.9	30.2	12.9	5.4	2.2	3.4						51.7	75.4
	Nonpregnant	89	2.2	23.6	28.1	30.3	10.1								25.8	4.6
India (Vellore)	Women : Pregnant	100	93.0	6.0	1.0	17.2	9.1	7.1		1.0					99.0	100.0
	Nonpregnant	99	16.2	26.3	2.0	12.1	30.3	33.3	17.2						42.5	60.7
	Men	99	5.1												5.1	7.1
Mexico	Women : Pregnant	126	30.2	31.0	12.7	18.3	6.3	0.8	0.8						61.2	71.5
	Nonpregnant	121	10.7	17.4	12.4	29.8	18.2	9.1	0.8	0.8	0.8	0.9			28.1	36.4
	Men	112	1.8	1.8	8.9	20.5	31.3	25.0	7.1	1.8	0.9	0.9			3.6	10.7
Venezuela	Women : Pregnant	62	40.3	19.4	12.9	21.0	4.8	1.6			0.9				59.7	69.4
	Nonpregnant	106	5.7	13.2	20.7	35.8	11.3	10.4	1.9						18.9	28.3
	Men	49			6.1	22.4	46.9	20.4	2.0	2.0						2.0

TABLE 6. PACKED CELL VOLUME

Country	Subjects	Total number of cases	Proportion (%) of cases with the following packed cell volumes (%)												Proportion of cases with anaemia (%)		
			< 21	22-23	24-25	26-27	28-29	30-31	32-33	34-35	36-37	38-39	40-41	42-43		44-45	≥ 46
Israel	Women : Pregnant Nonpregnant Men	98 99 65				2.0	5.1	12.3 1.0	19.4 1.0	21.4 2.0 1.5	19.4 4.0	11.2 19.2 1.5	7.1 22.2 7.7	2.0 27.3 20.0	17.2 32.3	6.1 36.9	29.6 4.0 3.0
Poland	Women, pregnant	220					1.8	4.6	12.3	15.0	20.4	22.8	17.7	3.6	0.5	1.4	11.4
India (Delhi)	Women : Pregnant Nonpregnant	100 95	1.0 4.2	1.0	2.0 3.2	7.0 6.3	12.0 5.3	14.0 4.3	17.0 6.3	27.0 13.7	6.0 20.0	6.0 22.1	6.0 9.5	3.2	1.0 1.1	1.1	43.0 43.3
India (Vellore)	Women : Pregnant Nonpregnant Men	100 100 99	10.0	4.0		7.0 2.0 1.0	8.0 1.0	15.0 3.0	20.0 9.0	12.0 7.0 1.0	11.0 21.0 1.0	10.0 27.0 4.0	1.0 16.0 8.1	1.0 12.0 18.2	1.0 1.0 16.2	1.0 50.5	52.0 22.0 3.0
Mexico	Women : Pregnant Nonpregnant Men	124 111	0.8 0.9	0.9	0.9	0.8 0.9	0.9	4.0 2.7	2.4 0.9	8.1 2.7	11.3 2.7	24.2 1.8	18.5 6.3 0.9	14.5 12.6 1.8	8.9 28.8 7.3	6.5 41.4 90.0	16.1 10.8 0.9
Venezuela	Women : Pregnant Nonpregnant Men	95 106 52			1.1			6.3 1.9 1.9	17.9 1.8 1.9	16.9	14.7 1.9	19.0 6.6	17.9 28.3 1.9	3.2 33.0 5.8	2.1 18.9 11.5	1.1 7.5 76.9	16.9 3.7 3.8

TABLE 7. SERUM FOLATE \*

Country	Subjects	Total number of cases	Proportion (%) of cases with the following serum folate levels (ng/ml)			
			< 3.0	3.0-5.9	6.0-19.9	≥ 20.0
Israel	Women :					
	Pregnant	64	6.3	42.2	51.6	
	Nonpregnant	99	5.1	30.3	62.6	2.0
	Men	61	1.6	13.1	78.7	6.6
Poland	Women, pregnant	212	1.4	14.6	60.8	23.1
India (Vellore)	Women :					
	Pregnant	100	9.0	61.0	30.0	
	Nonpregnant	100		25.0	71.0	4.0
	Men	99	2.0	22.2	75.8	
Mexico	Women :					
	Pregnant	124	6.5	54.0	37.9	1.6
	Nonpregnant	116	6.0	44.0	44.0	6.0
	Men	113	3.5	44.2	44.2	8.0
Venezuela	Women :					
	Pregnant	93	15.1	46.2	38.7	
	Nonpregnant	105	9.5	44.8	41.9	3.8
	Men	48	18.8	39.6	41.7	

\* Assayed by *Lactobacillus casei*.

TABLE 8. SERUM VITAMIN B<sub>12</sub>

Country	Subjects	Total number of cases	Proportion (%) of cases with the following serum vitamin B <sub>12</sub> levels (pg/ml)			
			< 80	80-139	140-199	≥ 200
Israel	Women :					
	Pregnant	50	2.0	6.0	36.0	56.0
	Nonpregnant	98		1.0	1.0	98.0
	Men	54				100.0
Poland	Women, pregnant	210	0.5	7.1	21.4	71.0
India (Delhi)	Women :					
	Pregnant	100	49.0	31.0	12.0	8.0
	Nonpregnant	90	26.7	41.1	21.1	11.1
India (Vellore)	Women :					
	Pregnant	100		49.0	40.0	11.0
	Nonpregnant	100	3.0	16.0	46.0	35.0
	Men	99		25.3	32.3	42.4
Mexico	Women :					
	Pregnant	85	7.1	8.2	3.5	81.2
	Nonpregnant	114			2.6	97.4
	Men	93	1.1		4.3	94.6
Venezuela	Women :					
	Pregnant	87	23.0	19.5	23.0	34.5
	Nonpregnant	100	1.0	2.0	6.0	91.0
	Men	49	2.0		10.2	87.8

TABLE 9. PREVALENCE OF ANAEMIA AND OF IRON, VITAMIN B<sub>12</sub>, AND FOLATE DEFICIENCY

Country	Subjects	Proportion of cases with anaemia <sup>a</sup> (%)	Proportion (%) of cases with the following deficiencies			
			Serum iron < 50 µg/100 ml	Transferrin saturation < 15 %	Serum vitamin B <sub>12</sub> < 80 pg/ml	Serum folate < 3 ng/ml
Israel	Women :					
	Pregnant	47.0	36.0	46.3	2.0	6.3
	Nonpregnant	29.0	15.0	11.4	0	5.1
	Men	13.6	12.1	8.8	0	1.6
Poland	Women, pregnant	21.8	31.4	40.0	0.5	1.4
India (Delhi)	Women :					
	Pregnant	80.0	10.0	51.7	49.0	
	Nonpregnant	64.3	9.5	25.8	26.7	
India (Vellore)	Women :					
	Pregnant	56.0	99.0	99.0	0	9.0
	Nonpregnant	35.0	51.0	42.5	3.0	0
	Men	6.0	7.1	5.1	0	2.0
Mexico	Women :					
	Pregnant	26.6	30.5	61.2	7.1	6.5
	Nonpregnant	11.7	22.1	28.1	0	6.0
	Men	0.9	6.2	3.6	1.1	3.5
Venezuela	Women :					
	Pregnant	37.0	56.8	59.7	23.0	15.1
	Nonpregnant	14.9	14.0	18.9	1.0	9.5
	Men	1.9	7.5	0	2.0	18.8

<sup>a</sup> As diagnosed by the haemoglobin levels given on p. 9 (with the exception of Mexico, for which the levels were increased by 1 g/100 ml to correct for altitude).

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