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NUTRITIONAL ANAEMIAS

Report of a WHO Group of Experts

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Geneva, 11-15 October 1971

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NUTRITIONAL ANAEMIAS

Report of a WHO Group of Experts

A WHO Group of Experts on Nutritional Anaemias met in Geneva from 11 to 15 October 1971.

The meeting was opened by Dr H. Mahler, Assistant Director-General, on behalf of the Director-General.

1. INTRODUCTION

The Group examined the validity of parameters and concepts in the field of nutritional anaemia and reviewed the information that had become available since the meeting of the Scientific Group on Nutritional Anaemias in 1967. The subjects covered by this review included the standardization of techniques; studies on the availability and absorption of iron, folate, and vitamin B₁₂; and prevalence studies and trials of preventive measures in population groups.

For the purposes of the meeting and of the present report, the terminology given in Annex 1 was adopted.

2. GENERAL CONSIDERATIONS

Normal human beings have stores of iron, folate, and vitamin B₁₂. If these are slightly reduced, no clinical or biochemical abnormality may result, but the ability to meet increased demands for nutrients (e.g., during pregnancy) is decreased. A further depletion of these stores may produce biochemical and/or clinical effects, but not necessarily anaemia, whereas yet a further reduction results in anaemia.

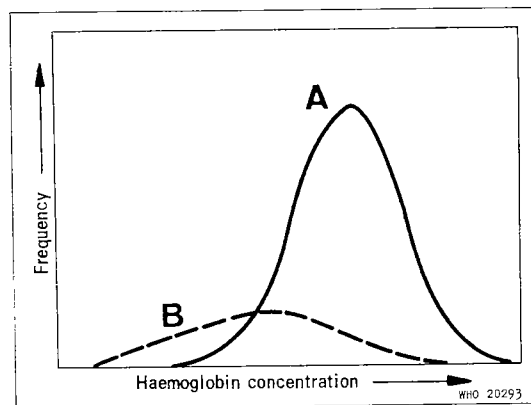
It is very difficult to quantify "normal" stores and to define deficiency. At present, the plasma iron, iron-binding capacity, and mean corpuscular haemoglobin concentration provide the most practical means of recognizing iron deficiency. With further developments in methodology it is possible that estimations of red cell protoporphyrin levels may also be helpful. Estimations of serum vitamin B₁₂ and of folate in serum red cells and tissues enable subjects likely to be deficient in these nutrients to be identified. Values below which deficiency is probably present are shown in Annex 2. The effects of these deficiencies — apart from that of making the affected

individual more liable to develop anaemia — are difficult to evaluate. Clearly anaemia is only a late manifestation of such deficiencies, but since it is easily detected and quantified it has received the most attention so far.

The definition of “normal haemoglobin concentration” is difficult. It is recognized that there is a homeostatic mechanism that sets the haemoglobin 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 distribution of such normal values in the population should be derived from a representative sample of healthy persons in whom the presence of nutritional deficiencies has been excluded by specific laboratory determinations¹ or by the prior administration of haematinics.² 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.

Anaemia is defined as a condition in which the concentration of haemoglobin is below the level that is normal for a given individual. Nutritional anaemia results from a deficiency in one or more essential nutrients, regardless of the cause of the deficiency.³ In any community in which anaemia is prevalent, the distribution of haemoglobin concentrations in anaemic persons overlaps that for persons with normal haemoglobin concentrations⁴ (Fig. 1). It is clear, therefore, that there is no value for haemoglobin

FIG. 1. THEORETICAL FREQUENCY DISTRIBUTION CURVES FOR HAEMOGLOBIN CONCENTRATIONS IN (A) SUBJECTS WITH NORMAL HAEMOGLOBIN LEVELS AND (B) SUBJECTS WITH ANAEMIA



concentration that will separate anaemic from normal subjects with certainty. In the past, as a practical approach to the characterization of anaemia, it was customary to define the concentration of haemoglobin below which anaemia may be said to exist (Annex 2), but this is obviously an over-simplification. In studies of nutritional anaemias, it is considered

preferable to characterize the status of populations by providing frequency distributions rather than by relating it to a single arbitrary value. From such information, the probability that anaemia is present at a given haemoglobin concentration may be assessed.

The presence of mild anaemia resulting from a nutritional deficiency in a population can frequently be disclosed only by demonstrating increases in the haemoglobin concentrations following therapy.² Whether mild or moderate, anaemia *per se* is not associated with a detectable increase in morbidity or with easily measurable impairment of body function.⁵ In view of the reserve in the oxygen transport system and the capacity of the body to compensate for any impairment of one of its transport components, this is not surprising. Clearly anaemia cannot be equated with hypoxia at ordinary levels of activity, but it does reduce maximum oxygen transport.⁶ The relationship between health and nutritional deficiencies — with or without anaemia — deserves further study.

The theoretical objective of public health programmes in relation to nutritional anaemia is to eliminate the condition and ensure that all individuals have normal stores of haemopoietic nutrients. However, recommendations for achieving this should be based on adequate scientific information; the measures proposed should be technically and economically feasible; and there should be no doubt that their implementation will lead to a significant improvement in health and satisfy an actual need, so that scarce resources will not be wasted.

3. METHODOLOGY

Standardization of laboratory procedures

The Group considered problems involved in standardizing laboratory procedures for collaborative studies and for individual clinical laboratories. The objectives of WHO as regards standardization include (1) the development of satisfactory methods by the participating laboratories; (2) the provision of reference standards; (3) quality control of laboratory methods in WHO-sponsored collaborative studies; (4) the training of technicians; and (5) the dissemination of information. In view of continuing problems and requirements, it is hoped that WHO will continue its activities in this field and collaborate with other bodies, such as the International Committee of Standardization in Haematology (ICSH).

Haemoglobin and packed cell volume

Methods for these determinations are now well standardized.⁷

Serum iron

The WHO International Reference Centre for Anaemias, School of Medicine, University of Washington, Seattle, USA, has co-ordinated the work of collaborating laboratories and has recommended assay methods. At approximately 3-monthly intervals during the past 4 years, lyophilized sera and iron solutions have been distributed. Although this has led to some improvement in the consistency of results, divergencies between laboratories are still wider than is considered acceptable for collaborative studies. Data studied by the Group indicate that, if all laboratories used chemical reagents from a single source, interlaboratory reproducibility might be improved.⁸

In 1967,³ it was recommended that the WHO reference centre should maintain contact with the ICSH Expert Panel on Iron. This panel has investigated techniques of serum iron assay and has recommended a method and a reference preparation.⁹ An experimental batch of standard reference material, based on these recommendations, has been prepared at the International Laboratory for Biological Standards, Mill Hill, London.

The ICSH Expert Panel demonstrated a high degree of reproducibility within and between laboratories and both the method and reference preparation are currently being used in WHO laboratories and in field trials.¹⁰

Transferrin

Although satisfactory reproducibility has been achieved in individual laboratories, using frozen specimens, interlaboratory variation in estimates of iron-binding capacity, measured in lyophilized specimens, is still a problem. The ICSH Expert Panel is studying this problem with particular attention to certain details of the method in use and to the changes that occur in serum during lyophilization and storage.

The MgCO_3 method¹¹ may be recommended, but it is not applicable in its present form to lyophilized material.

Erythrocyte protoporphyrin

It has been known for some time that the concentration of red cell protoporphyrin provides a measure of the adequacy of iron supply to erythrocyte precursors. This assay may have advantages over other parameters in current use, such as the percentage saturation of transferrin. A simplified method of assay has recently become available¹² and its applicability to the study of nutritional anaemias should be investigated further.

Folate and vitamin B₁₂

The WHO Regional Reference Centre for Anaemias, Department of Pathology, St Bartholomew's Hospital Medical College, London, England,

has sent samples of lyophilized human serum at regular intervals to the collaborating laboratories and to a number of workers, many of whom have originated assay methods. The vitamin B₁₂ and folate in lyophilized fresh serum was found to be stable for more than 2 years at 4 °C and for at least 2 months in the dark at room temperature.

There was good agreement within and between laboratories in the results of the *Lactobacillus casei* folate assay. However, in view of the complexity of the assay, constant monitoring and the regular use of reference preparations are necessary.

As for the results of serum vitamin B₁₂ assays, there was agreement between the results from the laboratories using *Euglena gracilis*,^{13,14} as well as between those obtained by laboratories in which small amounts (0.1 ml) of vitamin B₁₂ free serum were added to the aqueous standards of the assay. When this was not done, the serum levels were lower and more variable, but were not so affected as to invalidate the diagnostic use of the assay. It was concluded that this assay can be accepted for standard use.

The results of the *Lactobacillus leishmannii* assay, although perhaps more variable, were similar to those for *E. gracilis* when sera with normal or very low vitamin B₁₂ levels were assayed. They were much more variable when the vitamin B₁₂ levels were in the range 60–200 pg per ml. This assay is adequate for diagnostic purposes but is not recommended for research purposes.

Not enough results are yet available to justify comment on the *Escherichia coli* assay.

Vitamin B₁₂ assays employing radioactive isotope techniques gave results that were much more variable and tended to show higher levels than the corresponding microbiological assays. Of the existing methods, the one introduced by Tibbling¹⁵ gave the results closest to those obtained with *E. gracilis*. It is doubtful if a radioactive isotope assay is satisfactory for general use at this stage.

Standardization of survey methods

Studies of populations must be based on satisfactory epidemiological techniques. Such techniques have been described in the report of a WHO Expert Committee.⁷⁹ It may not always be possible to apply ideal methods, and any procedure that departs from the ideal should be clearly stated as such in published reports.

In nutritional studies the procedure for selecting the population sample to be studied is of fundamental importance and has often received inadequate attention. Only a sample drawn at random from a well defined total population can be assumed to be representative. Thus samples of subjects selected, for example, by area of residence, place of contact, or convenience to the investigator are not representative. In order to ensure

that a sample remains representative after selection, omissions for technical reasons, refusal to co-operate, etc., must be kept to a minimum and reported. Hospital patients are among the least satisfactory groups for the estimation of any population parameter.

Careful attention must always be paid to the conditions under which subjects are examined and samples for analysis are obtained. As standardization of field studies may be difficult, it is important that, when examinations are made and specimens obtained, all relevant circumstances — e.g., the hour, the time that has elapsed since food and exercise were last taken — should be accurately noted.

4. PREVALENCE STUDIES

There have probably been adequate studies of the prevalence of anaemia in Great Britain,^{16,17} Sweden,^{2,18} and a few other countries.¹⁹ On the other hand, in many areas, including developing countries, there are very few epidemiological data on any aspect of nutritional anaemias. Further prevalence studies are needed, but careful attention must be paid to methods of population sampling.

Iron deficiency

Studies presented to the WHO Scientific Group on Nutritional Anaemias in 1967³ indicated that, in several countries, anaemia and iron deficiency were highly prevalent, particularly in pregnant women. This has been confirmed by more recent reports²⁰ and by collaborative investigation in 7 Latin American countries, sponsored by WHO and PAHO.²¹

Previous reports that in some countries there is a high prevalence of iron deficiency among infants and young children of low social and economic status were also confirmed.^{22, 23, 24, 25} One study, conducted in Mexico,⁸ showed this to be true even in infants who had no evidence of illness and had been having a balanced diet containing fortified cereals that gave them 1.5–2.0 mg of iron per kg of body weight per day during the first year of life. However, the deficiency is a temporary one, disappearing after the age of 2 or 3 years without iron supplementation. This is confirmed by a report from Israel,²⁶ where anaemia was found in 52% of 247 healthy children, aged 1 day to 6 years, who were examined in Kiryat Shmoneh. Subnormal serum iron concentrations and whole-blood folate activity were found in 41% and 53% of these children, respectively. The prevalence of the anaemia and of the iron and folate deficiencies rose from birth to the age of 2–3 years, after which it declined gradually. The clinical significance of these temporary deficiencies is not known.

Folate and vitamin B₁₂ deficiency

Laboratory methods for folate and vitamin B₁₂ estimation that are suitable for use on a large scale in community surveys have become available only relatively recently. As a result, few prevalence surveys of folate and vitamin B₁₂ deficiency have been undertaken, although there have been several reports on this condition in hospital patients, selected samples of pregnant women, and inmates of institutions such as old people's homes.

Recent surveys in representative samples of apparently healthy elderly persons in Great Britain^{27, 28} have detected about 10–15% with folate or vitamin B₁₂ levels suggestive of deficiency. However, these findings²⁹ are difficult to interpret, since clear evidence of megaloblastic anaemia was found in well under 1% of the study population. More data are urgently needed on the clinical significance of low levels of folate and vitamin B₁₂ in non-pregnant subjects without evidence of other haematological changes. The few published studies of such persons have failed to detect any impairment of health,³⁰ and it appears that low levels of vitamin B₁₂ may persist for years without producing illness.³¹

Studies on the prevalence of folate and vitamin B₁₂ deficiencies and of megaloblastic changes, in selected groups of pregnant women in certain countries, were reviewed in 1967.³ More recent reports have confirmed the observation that low serum folate concentrations are very common in late pregnancy, and in a number of studies these have been shown to be accompanied by a fall in red cell folate concentrations. It has been asserted that folate deficiency in pregnancy may be associated with an increased prevalence of a variety of obstetric conditions such as abruptio placentae, abortion, fetal malformation, stillbirth, neonatal death, low birth weight, prematurity, toxæmia, and postpartum haemorrhage. However, most of these relationships have not been adequately established.³²

5. THE OCCURRENCE AND ABSORPTION OF HAEMOPOIETIC NUTRIENTS

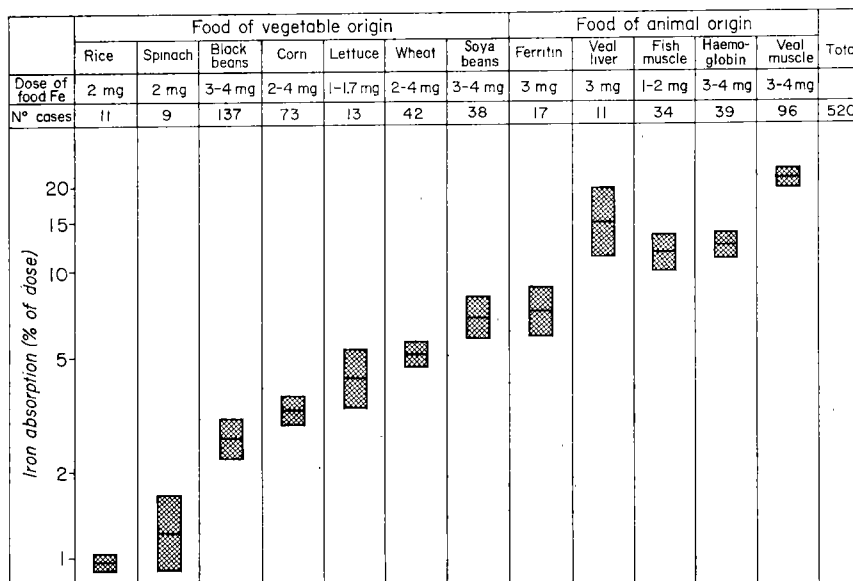
Iron

Problems connected with the absorption of food iron have been considered in previous reports.^{3, 33} It was then apparent that the amount of iron absorbed by the body depended on several factors, including the total amount in the diet, its absorbability, and the regulation of its absorption by the body. However, more precise information was needed about the effects on iron absorption of interactions between various dietary constituents. To this end, joint meetings were arranged by IAEA and WHO so that research could be co-ordinated.

Absorption from single foods

Studies³⁴ of the absorption of biosynthetically labelled foodstuffs in 524 persons, half of whom were deficient in iron, indicated that the mean absorption of iron from vegetable foods ranges from 1% for rice and spinach to 6% for soya beans, with intermediate values of 3% for maize and black beans, 4% for lettuce, and 5% for wheat. Mean iron absorption from animal foods ranges from 7% for ferritin to 22% for veal muscle, with intermediate values of 11% for fish, 12% for haemoglobin, and 13% for liver (Fig. 2). These results agree with those reported previously³⁵ for

FIG. 2. IRON ABSORPTION FROM FOOD *



* Collaborative study of the Departments of Botany and Medicine, University of Washington at Seattle, USA, and the Department of Pathophysiology, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela. The horizontal thick line represents the geometrical mean and the cross-hatched area shows the limits of one standard error.

soya beans, which gave lower figures than were initially reported. The difference may be due to changes in the preparation of the soya beans (temperature and duration of cooking), and this is being investigated. Limited studies in infants indicate that their absorption of iron from vegetable foodstuffs is of the same order as in adults.

Effects of interaction between foods

There is evidence that the absorption of iron from one foodstuff may be influenced by the simultaneous administration of other foods.^{36, 37, 38}

Meat increased the absorption of iron from a number of sources, including maize, black beans, haemoglobin, and inorganic iron salts. Cysteine has been shown to be one of the substances responsible for this enhancing effect. Administration of ascorbic acid increases absorption from non-haem food iron, whereas phytates and eggs cause a marked reduction. Geophagia depresses iron absorption and may be a more common cause of iron deficiency in some countries than has previously been realized.³⁹

Absorption from the whole diet

It has been suggested that the absorption of food iron takes place from 2 independent pools, a haem iron pool and a non-haem iron pool, and that the absorption from food could be estimated by the use of extrinsic tracers — a radioactive iron salt for the non-haem iron pool and radioactive haemoglobin for the haem iron pool.^{40, 41, 42} Evidence validating this technique was discussed by the Group.

In studies of non-haem iron, a number of investigators have found an absorption ratio (extrinsic/intrinsic tracers) close to 1 for a variety of foods (maize, white flour, wheat, bran, soya beans, and eggs) over a wide range of absorption rates.

The simultaneous administration of desferrioxamine reduces the absorption of both the extrinsic and intrinsic tracers to the same extent, and ascorbic acid and meat increase both.

In studies of haem iron, 2 groups of workers have found an absorption ratio (extrinsic to intrinsic tracer) of about 1.1 when the radioactive haemoglobin was mixed, before cooking, with intrinsically labelled minced veal.

It appears to be possible, therefore, to study the absorption of iron from a complete meal in which the non-haem pool of food iron is labelled with a radioactive iron salt and the haem pool is labelled with haemoglobin containing another isotope of iron.^{40, 41} Results obtained with this method suggest that the values found were compatible with other indirect data on the magnitude of iron absorption under different conditions. The results further indicate that the absorption of iron from a mixed "Western type" diet is approximately 6% in normal males, 14% in normal females, and 20% in iron-deficient subjects. In contrast, even in persons who are deficient in iron, very much less iron is absorbed from predominantly cereal diets. However, before applying this method on a wider scale, it is necessary to study various factors, such as the effects of gastric acidity, the bulk of the diet, mixing techniques, and cooking procedures.

Folate

Occurrence of folate

Data on the occurrence of folate in foods were reviewed earlier.³³ Perhaps the most valuable reference is that to the work of Toepfer et al.,⁴³

who list the folate content of some 348 foods. Unfortunately this work appeared over 20 years ago, before it was recognized that higher values are obtained if ascorbate is used to prevent loss of heat-labile forms. There is great need for an authoritative compendium of food folate values, prepared by more modern methods. Studies of the folate content of foods call for standardization of methods and for a technique to confirm the activity of enzyme preparations and the completeness of the conversion of polyglutamates to forms available to the assay organism.

In some foods, folate may be bound to other substances. In vegetables, there is evidence that folic acid is non-specifically bound to cellulose fibres.^{44, 45} Diets subjected to conjugase treatment after the removal of fibres appear to contain several times as much folate as crude homogenates do. The importance of this phenomenon has not been evaluated in terms of the availability of folate for human beings. Both human milk and cow's milk contain approximately 50 µg of total folate per litre⁴⁶ bound to proteins related to β-lactoglobulins.⁴⁷ The bond is destroyed by heat, but it is not known to what extent digestion renders the folate available for absorption by infants. This protein presumably accounts for the incorporation of folate by mammary tissues even in preference to the haemopoietic system, and helps to explain the increased folate requirements during lactation.

Folate absorption and utilization

Earlier investigations were largely confined to the study of pteroylmonoglutamate absorption, and are of doubtful relevance to the absorption of food folate.

The introduction of labelled polyglutamates⁴⁸ provided an important aid to research. It has been shown that, when the ¹⁴C label was in the pteroyl moiety, or in the first glutamic acid, the oral administration of polyglutamates caused ¹⁴C-labelled pteroylmonoglutamate to appear in the plasma. However, when the label was in the second or subsequent glutamic acids, no radioactivity appeared in the plasma, although the exhaled CO₂ was radioactive. This is strong evidence that, in the process of absorption, the polyglutamates are usually split into monoglutamates, presumably by the action of conjugase present in the intestinal juice and mucosal absorptive cells. It is not yet clear whether polyglutamates are always transformed into monoglutamates before absorption, or whether some of them (particularly di- and tri-glutamate forms) may be absorbed direct as such. When polyglutamate forms of folate are ingested, plasma levels of free folate rise but a rise in conjugated forms of folate has not been found.⁴⁹ Plasma itself contains a powerful conjugase that has maximal activity *in vitro* at an acid pH. If this enzyme is active *in vivo* at the pH of plasma, the failure to observe conjugated forms in the circulation cannot necessarily be taken as evidence of the lack of absorption of polyglutamate forms.

In the past, it was thought that only the "free" component of dietary folate was available for meeting nutritional requirements. However, there is now evidence that pteroylpolyglutamates are nutritionally active in man,⁴⁸ so that both "free" and "bound" forms of folate should be considered to be available. In metabolic studies in man⁵⁰ it has been observed that, after the ingestion of folic acid, plasma levels rise rapidly, faecal losses are low, and urinary excretion is high. As the number of glutamate residues in the side chain of synthetic polyglutamates increases, the faecal losses are greater, plasma levels rise more slowly, and urinary excretion decreases. After the ingestion of either folic acid or pteroylheptaglutamate, net losses from the body appear to be about 50% of the amount ingested. In other words, it appears that approximately half of the folate is retained and half of it is excreted whether it is ingested in a polyglutamate or in a monoglutamate form.

Factors that may interfere with folate absorption

It is well known that megaloblastic anaemia may occur with the use of certain anticonvulsant drugs, such as diphenylhydantoin. It has been suggested that this drug interferes with the intestinal deconjugation of dietary folate.⁵¹ However, other investigators have not been able to confirm such a mechanism of action.⁵² It has also been suggested that oral contraceptive agents may induce megaloblastic anaemia in certain cases by interfering with folate absorption, but further information is needed on this point. There is also evidence⁵³ that alcohol, in addition to increasing folate requirements, may interfere with folate absorption. Diseases of the small intestine, such as gluten-induced enteropathy and tropical sprue, also may interfere with folate absorption.

Vitamin B₁₂

Man derives most of his dietary vitamin B₁₂ from the flesh of ruminant animals or from dairy products, such as milk and cheese. The original synthesis of the vitamin occurs almost exclusively in micro-organisms. Other rich sources are certain molluscs and crustaceans that filter and scavenge micro-organisms from the surrounding water as part of their own food. There is little or no vitamin B₁₂ in plant foods grown under sterile conditions, but both these and drinking water may be contaminated with vitamin B₁₂ of bacterial origin.

The vitamin B₁₂ in food is usually bound to protein through peptide. The vitamin is released by the heat of cooking and by the action of gastric acid and gastric or intestinal enzymes. Apparently it is relatively stable to heat at the temperatures reached in food preparation.

Vitamin B₁₂ may exist with a variety of substituents attached to the cobalt atom. Cyanocobalamin is apparently an artefact of isolation,

although there is evidence that vitamin B₁₂ can accept -CN groups in nature and can protect against the toxicity of cyanogenic compounds.⁵⁴ This and other substituents, such as -OH, -Cl, -NO₂, -CH₃, =SO₄, and -5'-deoxyadenosine, all appear to be nutritionally active and subject to the normal mechanisms of absorption.

The vitamin B₁₂ content of diets varies with the proportion of animal foodstuffs. In one study, in the USA, it ranged from 1 to 85 µg daily.⁵⁵ Average vitamin B₁₂ values for high-cost, low-cost, and poor diets were 31.6, 16, and 2.7 µg per day, respectively.

Other estimates indicate that most American diets contain between 50 and 100 µg daily.^{56, 57} The average daily diet in Great Britain contains approximately 5 µg.⁵⁸ Little is known about the vitamin B₁₂ content of the diet in developing countries, but recent estimates from India show a total intake of 0.3 to 0.4 µg per day.⁵⁹

As the available reports on the vitamin B₁₂ content of foods show a wide variety of complex problems associated with the extraction and assay of cobalamins (some of which are thermolabile or photolabile) there is need for more information on this subject.

Some attention should be given to the form in which vitamin B₁₂ is ingested. At least in pharmacological doses, retention of hydroxocobalamin appears to be more efficient than that of cyanocobalamin is.^{60, 61} In healthy volunteers, the renal clearance of cyanocobalamin was essentially the same as that of inulin, whereas the excretion of hydroxocobalamin was approximately one-third that of inulin.⁶² In experimental animals, the storage of vitamin B₁₂ in the liver and kidney is impaired by suboptimal intakes of riboflavin, folic acid, and choline.⁶³ Thus, as is the case with synthetic polyglutamates, the net retention of a test dose of cyanocobalamin may be less than with the naturally occurring dietary form.

6. RECOMMENDED INTAKES

Iron

In 1970, an FAO/WHO Expert Group³³ recommended certain daily intakes of iron. The present group reconsidered these figures but did not think that there was sufficient evidence to justify changes in the recommendations. However, certain points need to be restated.

The recommended intakes of iron (Table 1) — which refer only to food iron, exclusive of contamination with particulate iron, e.g., from the soil — will be enough in all but 5% of cases. Since, in a given community, there will always be a range of iron intakes, in practice, the mean intake for adequately nourished individuals will be higher than that recommended. It is assumed that the requirements of calories and of all other nutrients

TABLE 1. 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
Infants 0-4 months	0.5	— ^b	— ^b	— ^b
Infants 5-12 months	0.7 ^c	7 ^c	5 ^c	4 ^c
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 ^c	12
Menstruating women ^d	2.8	28	19	14
Men	0.9	9	6	5
Pregnant women	}	See 1970 Joint Report, ³³ p. 52		
1st half of pregnancy				
2nd half of pregnancy				
Lactating women				

^a In populations eating virtually no foodstuffs of animal origin, the intakes need to be higher.

^b Breast-feeding is assumed to be adequate.

^c Figures recalculated subsequent to the publication of *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 452.

^d For non-menstruating women, the recommended intakes are the same as for men.

are fully met. In populations eating virtually no foodstuffs of animal origin, the intakes will need to be raised considerably. However, owing to the lack of information on this point, no recommended intakes are specified for such groups. Furthermore, the recommendations in Table 1 are not valid for populations in which abnormal conditions, such as hookworm infection or malabsorption, or habits such as clay eating, are prevalent.

In view of the prevalence of iron deficiency anaemia and the size of the recommended intakes in relation to the usual levels, there is a clear need to increase the dietary iron intake in many, if not most, populations. This might possibly be accomplished in some areas through food fortification programmes, "the alternatives being either the provision of direct iron supplements for vulnerable groups or a major change in dietary habits."⁶⁸

Folate

In adults maintained on a diet containing very small amounts of "free" folate and an ill-defined amount of total folate, it has been shown that a supplement of 50-100 µg of pteroylmonoglutamic acid is sufficient to meet daily requirements. But in fact both "free" and polyglutamate forms of folate are available from the diet approximately to the same extent. Such adults receive not only pteroylmonoglutamic acid: their diet also supplies them with an undetermined amount of polyglutamate forms of folate.

which should be taken into account in evaluating the requirements of total folate and not only those of "free" folate, as was done by the FAO/WHO Expert Group.³³ The values for requirements of total folate are given in Table 2. However, these requirements are increased during pregnancy,

TABLE 2. RECOMMENDED DAILY INTAKES OF FOLATE

Group	µg of total folate ^a
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

^a "Total folate" includes "free" and "bound" or polyglutamate forms.

lactation, hypermetabolic states, and chronic haemolytic disease, as well as in chronic alcoholism. In such situations, the requirement in terms of total folate is at least 600 µg daily.

The previously published figures for children under 6 months of age were based on estimates of the total folate content of milk and not just its "free" folate content. The figures therefore remain unchanged.

Vitamin B₁₂

Vitamin B₁₂ requirements were considered, as suggested by the Joint FAO/WHO Expert Group,³³ and, although in the opinion of some participants these amounts were greater than the minimum requirements for health, the Group did not think that enough new information had accumulated to justify any change in the recommendations (see Table 3).

TABLE 3. RECOMMENDED DAILY INTAKES OF VITAMIN B₁₂

Group	µg of vitamin B ₁₂
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

7. IRON AND FOLATE SUPPLEMENTATION IN PREGNANCY

The objective of nutrient supplementation during pregnancy is to ensure optimum health of mother and child. This implies that the additional nutritional requirements during pregnancy and lactation must be met and any pre-existing deficiencies must be corrected. The Group considered trials of supplementation carried out recently in India, Israel, and Mexico.

In Israel,⁶⁴ in a community of low socioeconomic status, the daily administration to pregnant women of iron (100 mg), folic acid (300 µg), and vitamin B₁₂ (100 µg) was found to cause a rise in the haemoglobin concentration of more than 0.5 g in 90% of the women. The combined administration of iron and folic acid was more effective than the administration of either of these alone. When every pregnant woman found to be anaemic was systematically treated with this combination of haematinics, the prevalence of anaemia in this group was reduced from over 50% in 1965 to 6% in 1970. However, even when all 3 haematinics were administered, the mean haemoglobin concentration in the women examined did not rise above 11 g per 100 ml.

In a study in Hyderabad, India,⁶⁵ 30 mg of iron were given daily during the last trimester of pregnancy to women with initial haemoglobin concentrations higher than 8.5 g per 100 ml. The haemoglobin concentrations rose in about 30% of the women and were maintained at their initial levels in a further 60%. The addition of folic acid (500 µg) and vitamin B₁₂ (2 µg) to this regimen produced no further elevation of haemoglobin.

Two WHO collaborative studies were carried out in Delhi⁶⁶ and Vellore, India.⁶⁷ Supplements were given for 10–12 weeks, starting at about the 26th week of pregnancy. Control groups received only placebo and showed a progressive fall in the haemoglobin level. The women whose diet was supplemented received vitamin B₁₂ (100 µg intramuscularly once a fortnight), folic acid (5 mg daily), and iron (in a single daily dose of 30, 60, 120, or 240 mg). In most of the women receiving iron there was a significant increase in the haemoglobin concentration, though little difference in efficacy could be detected between the different doses of iron. Even in the group receiving 240 mg of iron, the haemoglobin concentration did not rise above 11 g per 100 ml in about half of the women. Subjects receiving vitamin B₁₂ and folate alone, without added iron, showed a mean drop in the haemoglobin level similar to that in the group receiving only placebo.

In a study in Mexico⁸ pregnant women were given 1.2 g of iron dextran intravenously. Whatever the haemoglobin level was at the time of injection, it reached a mean value of 14.5 g per 100 ml at labour, provided that enough time had elapsed between the injection and labour. Therefore this level seems to be the homeostatic level for pregnant women living at an

altitude of 2 550 m above sea level (equivalent to about 13 g per 100 ml at sea level).

The reason why the anaemic pregnant women in these studies failed to achieve normal haemoglobin concentrations in spite of apparently adequate therapy is unknown. In Delhi and Vellore, the iron tablets were not administered while the subjects were fasting. It is therefore likely that the absorption of the iron was depressed by the food in the predominantly vegetarian diet. It is also possible that the low protein intake of these women (about 30 g of vegetable protein per day) may have limited their haemopoietic response.

In these, as well as earlier studies, it has been shown that low serum concentrations of folate and vitamin B₁₂ are prevalent. However, the relative importance of the deficiency in these nutrients cannot be evaluated from the present studies, although the results from Israel indicate that, at least in some communities, it is advisable to supplement the diet with folic acid as well as iron.

On the basis of the reported results of supplementation in pregnancy, and of recent data on iron absorption, the Group considered public health measures for the prevention of anaemia in pregnancy. Variations in the dietary intake of iron, and in iron stores, in different population groups make it necessary to recommend iron supplementation at different dosage levels (see Table 4). In women more than 25% of whose caloric intake

TABLE 4. RECOMMENDED AMOUNTS OF IRON AND FOLIC ACID SUPPLEMENTATION IN PREGNANCY

	Iron daily (mg)	Folic acid daily ^a (µg)
Populations with iron stores	30-60	500
Populations without iron stores	120-240	500

^a In severe folate deficiency, or in multiple pregnancies, more than 500 µg may be necessary.

comes from animal sources and who have iron stores at the beginning of pregnancy, a daily supplement of 30 mg of iron is sufficient to maintain optimum haemoglobin concentrations. However, there is evidence that these conditions seldom exist, even in developed countries, and it is probable that a daily supplement of 60 mg of iron is desirable. In countries where less than 10% of calories come from foods of animal origin, iron deficiency is prevalent and many women are anaemic at the commencement of pregnancy. Supplementation with greater amounts of iron is therefore required, and it is recommended that the dosage of iron in such circumstances should be 120-240 mg per day. All pregnant women should receive,

in addition to iron, supplements of folic acid of about 500 µg daily. Supplementation with both iron and folic acid should start not later than the second trimester of pregnancy and should continue until the end of lactation.

8. EVALUATION OF FOOD FORTIFICATION PROGRAMMES

Previous reports^{3, 33, 68} have shown that in some parts of the world it may be necessary to fortify foods with iron. The problem has been studied intensively during the past few years,^{34, 69, 70} but the resultant information has served more to emphasize the difficulties than to find solutions to them. It appears that 3 factors must be examined in any attempt to improve nutrition through fortifying foods with iron.

(1) *The availability of iron salts.* When an iron salt is being considered for use, its availability should be compared with that of ferrous sulfate, employing isotope techniques. Furthermore, its absorbability should be shown to be sufficiently good to justify its use. Some commonly used preparations are poorly absorbed.⁷⁰ In a recent study of 4 preparations (ferrous sulfate, ferrum reductum of small particle size, ferric orthophosphate, and sodium ferric pyrophosphate), only ferrous sulfate and ferrum reductum were effectively absorbed.⁷¹ Several studies on the fortification of foodstuffs with iron salts have been described. One study concerns the fortification of a fish sauce that is widely consumed in Thailand,⁷² the average consumption being about 15 ml a day. It has been possible to add 1 mg of elemental iron per ml as the EDTA salt, and preliminary results show that about 5% of this added iron is absorbed when the sauce is taken with the local diet. Other foodstuffs, such as salt and sugar, have been considered as possible vehicles for iron salts. A recent trial of fortified salt⁷³ gave disappointing results, almost certainly attributable to the nature of the iron compounds used and the cereal diet to which the fortified salt was added.

(2) *The availability of iron in a fortified food taken as part of the ordinary diet.* The above-mentioned studies on iron absorption have led to the hypothesis that all absorbable iron in a meal, whether natural food iron or added iron, enters one of two pools prior to absorption. If this is true, then "available" iron salts used to fortify a foodstuff will be absorbed to the same extent as the iron in the rest of the meal in which the fortified food is eaten, and are subject to the same enhancing factors (e.g., meat, ascorbic acid, cysteine) or inhibiting factors (e.g., phytate, eggs, bran). Whereas the identity of all the factors that may inhibit iron absorption is not known, there is already enough evidence to suggest that they may greatly limit the effectiveness of fortification programmes unless they can be eliminated or neutralized.⁷⁴ This problem is likely to be an important one especially in

countries such as India where, despite relatively high dietary intakes of iron, iron deficiency is common. The possibility of improving nutrition by employing substances that enhance iron absorption from foodstuffs should also be investigated. One promising approach⁷⁵ is based on the observation that, when relatively large amounts of ascorbic acid are added to cereals before these are cooked, not only does the absorption of the added iron increase significantly, but also that of the iron contained in the cereals. The availability of the iron in a fortified food included in a standard meal should determine the feasibility of a proposed fortification trial.

(3) *Evaluation of the effect of fortification on iron balance and health.* Since the fortification of food with iron is intended to prevent rather than correct iron deficiency, the best way to evaluate it would be to determine, in a representative population group, the extent to which a fortification programme prevents the development of iron deficiency. Such a study could be made in a population that had previously been found to be anaemic and that had been effectively treated with iron.⁷⁶ There is evidence that fortification of the milk and cereals given to babies protects them to some extent against the development of iron deficiency.^{77, 78} In adults the situation is more difficult, and fortification programmes have sometimes been carried out without adequate evaluation. Therefore it is recommended that any iron salt used in future field trials should be one that is reasonably well absorbed and is suitable for food fortification programmes. Furthermore, enough of the iron salt should be provided to ensure that an effective amount of it is absorbed from the basic diet. It is the effectiveness of the iron salt in preventing anaemia under ordinary living conditions that should be evaluated.

9. RECOMMENDATIONS

To ensure the comparability of studies by different investigators, and to improve diagnostic methods, laboratory procedures for the assessment of iron, folate, and vitamin B₁₂ nutrition should be developed further and standardized.

The assessment of dietary intakes requires more information on the content, nature, and absorbability of folate and vitamin B₁₂ in individual foodstuffs, and on the effects of processing, cooking, and storage. More knowledge is also needed regarding the absorption of iron, folate, and vitamin B₁₂ from common diets. In the case of iron this information can be obtained by means of the extrinsic tracer technique for measuring the absorption of the total iron contained in a meal.

Assessments of the prevalence and importance of anaemia and nutritional deficiencies, and of the effectiveness of preventive measures, must be based on epidemiological studies (see Annex 1) employing valid sampling proce-

dures and survey methods. These have been described in a previous WHO report.⁷⁹

The distribution of normal haemoglobin values (as defined in Annex 1) should be established in representative population samples. Since severe anaemia is known to be associated with increased morbidity and impaired function, it seems reasonable to assume that mild anaemia may also impair health. Controlled double blind trials of supplementation to eradicate anaemia should therefore be carried out, and the effects of eradication on indices of health such as morbidity ; growth and development ; and work capacity should be estimated.

Ideally, recommendations for public health action should be based on the results of such controlled double blind trials. Unfortunately, evidence of this kind is scarce and difficult to obtain, so current recommendations have to be based on estimates of prevalence. At the present time, public health action should be directed towards the elimination of anaemia in populations with a high prevalence. It is therefore recommended that, wherever possible, the diet of pregnant women should be supplemented as indicated in this report. At the same time, the effectiveness of such supplementation, carried out under normal conditions as a public health measure, should be carefully monitored by studying changes in haemoglobin concentrations and in morbidity and mortality in mothers and children. The recommended supplements are based on the best available evidence, but further information is necessary to establish the optimum combinations of haemopoietic nutrients required in different communities. The possible role of protein deficiency in the pathogenesis of anaemia in pregnancy also requires investigation.

The most practicable method of eliminating iron deficiency in a community is probably the fortification of food with iron. Suitable fortification procedures must therefore be developed, particular attention being paid to the form of iron employed ; the foodstuff to which it is added ; the amount of that foodstuff consumed by different segments of the population ; the adequacy of iron absorption from the fortified diet ; and the acceptability of the procedure to consumers and the food industry. Since the absorption of iron may be limited by the very nature of the diet, ways of enhancing it — e.g., by adding ascorbic acid — should also be investigated. The effectiveness of any fortification programme must be evaluated in controlled double blind field trials simulating normal conditions as closely as possible.

REFERENCES

1. Loria, A., Piedras, J., Labardini, J. & Sanchez-Medal, L. (1971) *Rev. Invest. clin.*, **23**, 3
2. Garby, L., Irnell, L. & Werner, I. (1969) *Acta med. scand.*, **185**, 113
3. WHO Scientific Group on Nutritional Anaemias (1968) *Wld Hlth Org. techn. Rep. Ser.*, No. 405
4. Pryce, J. D. (1960) *Lancet*, **2**, 333
5. Elwood, P. C. (1970) *Proc. roy. Soc. Med.*, **63**, 1230
6. Andersen, H. T. & Barkve, H. (1970) *Scand. J. clin. Lab. Invest.*, **25**, Suppl. 114
7. International Committee for Standardization in Haematology (1967) *Brit. J. Haemat.*, **13**, Suppl. 71
8. Sanchez-Medal, L. & Loria, A. (1971) Unpublished observations
9. International Committee for Standardization in Haematology (1971) *Brit. J. Haemat.*, **20**, 451
10. Conrad, M. E., Fielding, J. & Ramsay, W.N.M. (1972) Studies on the standardisation of serum iron and iron binding capacity assays. In : Izak, G. & Lewis, S.M., ed., *Modern concepts in haematology*, New York, Academic Press, p. 69
11. Ramsay, W. N. M. (1957) *Clin. chim. Acta*, **2**, 221
12. Heller, S. R., Labbe, R. F. & Nutter, J. (1971) *Clin. Chem.*, **17**, 525
13. Anderson, B. B. (1964) *J. clin. Path.*, **17**, 14
14. Anderson, B. B. (1965) *Investigations into the Euglena method of assay of vitamin B₁₂ : the results obtained in human serum and liver using an improved method of assay*, London (Thesis)
15. Tibbling, G. (1969) *Clin. chim. Acta*, **23**, 209
16. Elwood, P. C., Waters, W. E., Greene, W. J. & Wood, M. M. (1967) *Brit. med. J.*, **4**, 714
17. Jacobs, A., Waters, W. E., Campbell, H. & Barrow, A. (1969) *Brit. J. Haemat.*, **17**, 581
18. Hallberg, L., Hallgren, J., Hollender, A., Högdahl, A.-M. & Tibbling, G. (1968) *Occurrence of iron deficiency anaemia in Sweden*. In : Blix, G., ed., *Occurrence, causes and prevention of nutritional anaemias*, Stockholm, Almqvist & Wiksell, p. 19
19. Natvig, H., Vellar, O. D. & Andersen, J. (1967) *Acta med. scand.*, **182**, 183
20. Scott, D. E., Pritchard, J. A., Saltin, A. S. & Humphreys, J. M. (1970) *Iron deficiency during pregnancy*. In : Hallberg, L., Harwerth, H. G. & Vannotti, A., ed., *Iron deficiency*, London, Academic Press (Colloquia Geigy), p. 491
21. 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. & Viteri, F. (1971) *Blood*, **38**, 591
22. Apte, S. V. (1971) *Indian J. med. Res.*, **59**, Suppl. 49
23. Sanchez-Medal, L. (1969) *Iron deficiency in pregnancy and infancy*. In : *Iron metabolism and anaemia*, Washington, Pan American Health Organization (Scientific Publication No. 184), p. 65
24. Loria, A., Garcia-Viveros, J., Sanchez-Medal, L., Hoffs, M. D., Shein, M. & Berger, I. (1970) *Bol. méd. Hosp. infant. (Méx.)*, **27**, 251

25. Loria, A., Sanchez-Medal, L., Garcia-Viveros, J. & Piedras, J. (1971) *Rev. Invest. clin.*, **23**, 11
26. Levy, S., Hershko, C., Grossowicz, N., Rachmilewitz, M. & Izak, G. (1970) *Amer. J. clin. Nutr.*, **23**, 1364
27. Elwood, P. C., Shinton, N. K., Wilson, C. I. D. & Frazer, A. (1972) *Brit. J. Haemat.* (in press)
28. Berry, W. T. C., Mollin, D. et al. (1972) (in preparation)
29. Mollin, D. (1960) *Ann. Rev. Med.*, **11**, 333
30. Hughes, D., Elwood, P. C., Shinton, N. K. & Wrighton, R. J. (1970) *Brit. med. J.*, **2**, 458
31. Waters, W. E., Withey, J. L., Kilpatrick, G. S. & Wood, P. H. N. (1971) *Brit. J. Haemat.*, **20**, 521
32. Rothman, D. (1970) *Amer. J. Obstet. Gynec.*, **108**, 149
33. Joint FAO/WHO Expert Group on Requirements of Ascorbic Acid, Vitamin D, Vitamin B₁₂, Folate, and Iron (1970) *FAO Nutrition Meetings Report Series*, No. 47; *Wld Hlth Org. techn. Rep. Ser.*, No. 452
34. Layrisse, M. & Martinez-Torres, C. (1971) *Progr. Hemat.*, **7**, 137
35. Layrisse, M., Cook, J. D., Martinez-Torres, C., Roche, M., Kuhn, I. N., Walker, R. B. & Finch, C. A. (1969) *Blood*, **33**, 430
36. Layrisse, M., Martinez-Torres, C. & Roche, M. (1968) *Amer. J. clin. Nutr.*, **21**, 1175
37. Martinez-Torres, C. & Layrisse, M. (1970) *Blood*, **35**, 669
38. Martinez-Torres, C. & Layrisse, M. (1971) *Amer. J. clin. Nutr.*, **24**, 531
39. Moore, C. V. (1968) *The absorption of iron from foods*. In: Blix, G., ed., *Occurrence, causes and prevention of nutritional anaemias*, Stockholm, Almqvist & Wiksell, p. 92
40. Cook, J. D., Layrisse, M., Martinez-Torres, C., Walker, R., Monsen, E. & Finch, C. A. (1972) *J. clin. Invest.* (in press)
41. Layrisse, M. & Martinez-Torres, C. (1972) *Amer. J. clin. Nutr.* (in press)
42. Hallberg, L. & Bjorn-Rasmusen, E. (1972) *Scand. J. Haemat.* (in press)
43. Toepfer, E. W., Zook, E. G., Orr, M. L. & Richardson, L. R. (1951) *Folic acid content of foods*. Washington, D. C., United States Department of Agriculture (Agriculture Handbook No. 29)
44. Santini, R., Berger, F., Berdasco, G., Sheehy, T. W., Aviles, J. & Davila, I. (1962) *J. Amer. diet. Ass.*, **41**, 562
45. Luther, L. et al. (1965) *Ala. J. med. Sci.*, **2**, 389
46. Ford, J. E. & Scott, K. J. (1968) *J. Dairy Res.*, **35**, 85
47. Ford, J. E., Salter, D. N. & Scott, K. J. (1969) *J. Dairy Res.*, **36**, 435
48. Butterworth, C. E., Baugh, C. M. & Krumdieck, C. (1969) *J. clin. Invest.*, **48**, 1131
49. Rosenberg, I. H., Streiff, R. R., Godwin, H. A. & Castle, W. B. (1969) *New Engl. J. Med.*, **280**, 985
50. Butterworth, J. E. (1971) Unpublished observations
51. Druskin, M. S., Wallen, M. H. & Bonagura, L. (1962) *New Engl. J. Med.*, **267**, 483
52. Bernstein, L. H., Gutstein, S., Weiner, S. & Efron, G. (1970) *Amer. J. Med.*, **48**, 570
53. Halsted, C. H., Griggs, R. C. & Harris, J. W. (1967) *J. Lab. clin. Med.*, **69**, 116
54. Mushett, C. W., Kelley, K. L., Boxer, G. E. & Rickards, J. C. (1952) *Proc. Soc. exp. Biol. (N. Y.)*, **81**, 234

55. Chung, A. S. M., Pearson, W. N., Darby, W. J., Miller, O. N. & Goldsmith, G. A. (1961) *Amer. J. clin. Nutr.*, **9**, 573
56. Lichtenstein, H., Beloian, A. & Murphy, E. W. (1961) *Vitamin B₁₂ : microbiological assay methods and distribution in selected foods*, Washington, D. C., United States Department of Agriculture (Home Economics Research Report No. 13)
57. Corcino, J. J., Waxman, S. & Herbert, V. (1970) *Amer. J. Med.*, **48**, 562
58. Great Britain, Ministry of Agriculture, Fisheries and Food (1962) *Domestic food consumption and expenditure, 1960. Annual report of the National Food Survey Committee*, London, H. M. Stationery Office
59. Baker, S. J. & Mathan, V. I. (1971) Unpublished observations
60. Meyer, L. M., Schiffer, L. M., White, D. A. & Cronkite, E. P. (1965) *Brit. J. Haemat.*, **11**, 370
61. Kennedy, E. H. & Adams, J. F. (1965) *Clin. Sci.*, **29**, 417
62. Shearman, D. J. C., Calvert, J. A., Ala, F. A. & Girdwood, R. H. (1965) *Lancet*, **2**, 1328
63. Ellis, L. N., Duncan, B. J. & Snow, I. B. (1959) *J. Nutr.*, **67**, 185
64. Rachmilewitz, M. (1971) Unpublished observations
65. Iyengar, L. & Apte, S. V. (1970) *Amer. J. clin. Nutr.*, **23**, 725
66. Sood, S. K. (1971) Unpublished observations
67. Mathan, V. I., Swarnabai, C., Date, S. & Baker, S. J. (1971) Unpublished observations
68. Joint FAO/WMO Expert Committee on Nutrition (1971) *Eighth report. FAO Nutrition Meetings Report Series*, No. 49; *Wld Hlth Org. techn. Rep. Ser.*, No. 477
69. Elwood, P. C., Waters, W. E. & Sweetnam, P. (1971) *Clin. Sci.*, **40**, 31
70. Great Britain, Ministry of Health (1968) *Iron in flour*, London, H. M. Stationery Office (Reports on Public Health and Medical Subjects, No. 117)
71. Steinkamp, R., Dubach, R. & Moore, C. V. (1955) *Arch. intern. Med.*, **95**, 181
72. Garby, L. (1971) Unpublished observations
73. Rao, B. S. N., Prasad, S. & Apte, S. V. (1972) *Brit. J. Haemat.* (in press)
74. Layrisse, M., Martinez-Torres, C., Cook, J. & Finch, C. A. (1972) *Blood* (in press)
75. Sayers, M. H., Lynch, S. R., Jacobs, P., Charlton, R. W., Bothwell, T. H. & Mayet, F. G. H. (1971) Unpublished observations
76. Elwood, P. C., Waters, W. E. & Greene, W. J. W. (1970) *Lancet*, **2**, 175
77. Marsh, A., Long, H. & Stierwalt, E. (1959) *Pediatrics*, **24**, 404
78. United States, National Research Council, Food and Nutrition Board, Committee on Iron Nutritional Deficiencies (1970) *Workshop on measures to increase iron in foods and diets*, Washington, D. C., National Academy of Sciences
79. WHO Expert Committee on Health Statistics (1966) *Tenth report. Wld Hlth Org. techn. Rep. Ser.*, No. 336
80. Joint FAO/WHO Expert Group on Requirements of Vitamin A, Thiamine, Riboflavine and Niacin (1967) *FAO Nutrition Meetings Report Series*, No. 41; *Wld Hlth Org. techn. Rep. Ser.*, No. 362, p. 14

Annex 1

GLOSSARY

Anaemia. A reduction of the haemoglobin concentration, the haematocrit, or the number of red blood cells, to a level below that which is normal for a given individual (see *normal haemoglobin concentration*).

Deficiency, nutritional. A relative or absolute lack of a nutrient that, if it persists long enough and becomes severe enough, will lead to a deficiency disease.

Deficiency disease. Any specific pathological state, with characteristic clinical signs, that is due to an insufficient intake of energy or essential nutrients; it is usually of dietary origin and can often be prevented or cured by bringing the intake up to an adequate level.

Folacin. Synonym for *folate*.

Folate. A generic term, including all forms of heterocyclic compounds that are based on the pteric acid skeleton conjugated with one or more glutamic acid molecules in gamma peptide linkage and that are nutritionally active in man (synonym for *folacin*).

Folic acid. Pteroyl monoglutamic acid (PGA).

NOTE: This differs slightly from the definition given in the report of a FAO/WHO Expert Group,³³ in which *folic acid* and *folate* were used synonymously.

Fortification. Of the terms in current use (enrichment, restoration, etc), "fortification" was recommended in the eighth report of the Joint FAO/WHO Expert Committee on Nutrition as "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." ⁶⁸

Normal haemoglobin concentration. The haemoglobin concentration in a given individual under ideal conditions of health and nutrition. In a community, under ideal conditions of health and nutrition, the frequency distribution of the haemoglobin concentration in a representative population sample expresses the normal frequency distribution for the entire community.

Nutritional anaemia. A condition in which the haemoglobin concentration, the haematocrit, or the number of red blood cells is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency.³

Recommended daily intake. The daily amount of a nutrient considered to be sufficient for the maintenance of health in nearly all people.

NOTE: The recommended intake is not expected to cover any additional needs for the nutrient that "may result from abnormal conditions such as microbial and parasitic infections, malabsorption syndromes, or metabolic abnormalities of genetic or degenerative origin."⁸⁰ Some food additives, drugs, or other chemicals may also increase the requirements of essential nutrients. The recommended intake of each nutrient is established on the assumption that requirements of calories and all other nutrients are being fully met.

Vitamin B₁₂. One or more of a group of cobalamins that are nutritionally active for man, and whose essential chemical structure consists of a cobalt-containing tetrapyrrole (corrin) ring attached to a dimethylbenzimidazole nucleotide and an aminopropanol chain. "Cobalamin" may be used as a synonym for vitamin B₁₂.

A variety of substituents may be attached to the cobalt atom (-OH, -Cl, -NO₃, -CH₃, =SO₄, and -5'-deoxyadenosine) without materially affecting nutritional properties. Cyanocobalamin, thought to be an isolation artefact, is also nutritionally active in man. It has the advantage of being relatively heat-stable and is used as a reference standard.

NOTE: This definition is more complete than that given in a previous report.³³

Annex 2

INDICES SUGGESTIVE OF ANAEMIA AND
NUTRITIONAL DEFICIENCIES**Haemoglobin concentrations below which anaemia is likely to be present
at sea level**

	<i>g/100 ml</i>
Children 6 months to 6 years	11
Children 6–14 years	12
Adult males	13
Adult females, non-pregnant	12
Adult females, pregnant	11

Mean corpuscular haemoglobin concentration

Levels below 31 are likely to indicate iron deficiency

Serum iron concentrations and percentage saturation of transferrin

	<i>Normal range</i>	<i>Probable deficiency</i>
Serum iron ($\mu\text{g}/100\text{ ml}$)	80–180	< 50
Percentage saturation of transferrin	20–50	< 15

Serum vitamin B₁₂ and serum and red cell folate concentration

	<i>Normal range</i>	<i>Probable deficiency</i>
Serum B ₁₂ (pg/ml)	150–1000	< 100
Serum folate (ng/ml)	6–20	< 3
Red cell folate (ng/ml)	150–700	< 100