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Nutritional Anaemias

Report of a WHO Group of Experts

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

GENEVA
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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 B_{12}; 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

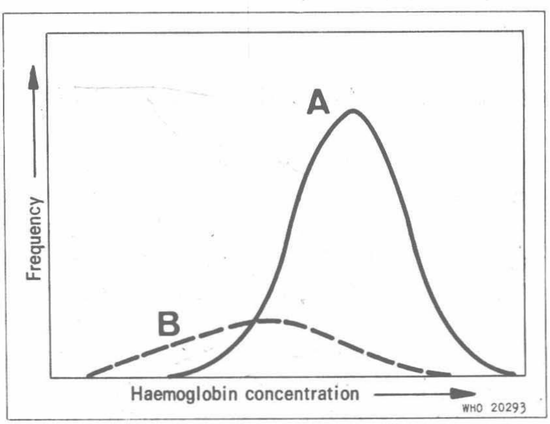
Normal human beings have stores of iron, folate, and vitamin B_{12}. If these are slightly reduced, no clinical or biochemical abnormality may result, but the ability to meet increased demands for nutrients (e.g., during pregnancy) is decreased. A further depletion of these stores may produce biochemical 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 B_{12} 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 homoeostatic 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 determinations [1] 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 concentrations [4] (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 collaboratives studies and for individual clinical laboratories. The objectives of WHO as regards standardization include (1) the development of satisfactoy 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 recommeded 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 $MgCO_3$ method [11] 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 available [12] and its applicability to the study of nutritional anaemias should be investigated further.

Folate and vitamin B_{12}

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 B_{12} 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 B_{12} 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 B_{12} 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 B_{12} levels were assayed. They were much more variable when the vitamin B_{12} 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 B_{12} 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 Tibbling [15] 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 1967 [3] indicated that, in several countries, anaemia and iron deficiency were highly prevalent, particularly in pregnant women. This has been confirmed by more recent reports [20] 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 B_{12} deficiency

Laboratory methods for folate and vitamin B_{12} 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 B_{12} 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 B_{12} levels suggestive of deficiency. However, these findings [29] 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 B_{12} 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 B_{12} may persist for years without producing illness.[31]

Studies on the prevalence of folate and vitamin B_{12} 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, toxaemia, and postpartum haemorrhage. However, most of these relationships have not been adequately established.[32]

5. THE OCCURRENCE AND ABSORPTION OF HAEMOPOIETIC NUTRIENTS

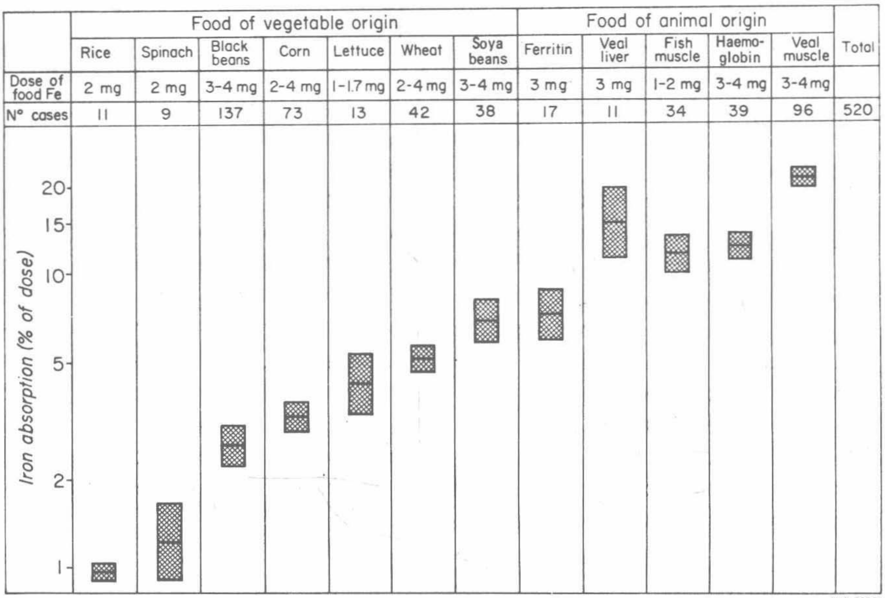
Iron

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

Studies[34] 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 previously[35] 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 Científicas, 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]