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ANEMIA AND NORMALITY

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INTRODUCTION

The automation of diagnostic laboratory procedures has made it possible to extend the scope of clinical assessment to the community outside the hospital. Fundamental to the interpretation of each observation is a knowledge of the range of values which should be considered normal for the person concerned. "Normal" values for haemoglobin, as for other body constituents, have frequently been determined from testing only a small number of persons in a single laboratory, or by accepting and applying the published results of other workers, often out of context, especially in relation to the comparability of methods used. Widespread use of automated techniques should make it possible to re-assess normal values for haemoglobin and other body constituents, making use of large numbers of observations and also of improvements and standardisation of methodology. The purpose of this paper is to review the published criteria of normality for the haemoglobin concentration, rather than propose another new arbitrary normal range for this parameter. In particular, the importance of careful selection criteria will be discussed, especially in relation to the exclusion of latent deficiencies of iron, vitamin B_{12} and folate, which may reduce the Hb concentration below its optimal level in otherwise apparently healthy subjects. The functional significance of a sub-optimal Hb concentration will also be discussed in relation to adaptive mechanisms which compensate for the reduced oxygen-carrying capacity of the blood.

DEFINITION OF ANAEMIA

In clinical medicine anaemia is defined as a reduction of the haemoglobin concentration below "normal". This is a functional definition based on the oxygen-carrying capacity of the blood. It can be argued whether the Hb concentration or the total red cell mass is the most valid index of anaemia. However, the former is more directly related to tissue oxygen supply and is certainly the only practical clinical measurement. The packed cell volume (PCV or haematocrit) can be used as an indirect screening test for anaemia, and in practice this measurement is usually made in conjunction with the Hb concentration, from which the MCHC (Hb g% x 100/PCV) can be calculated.

NORMAL Hb CONCENTRATION

The term "normal" is a statistical concept reflecting biological variability, and refers to the range of Hb values obtained in a random sample of apparently healthy subjects. However, what we really want to know is the "optimal" Hb concentration, which is a physiological concept implying maximal performance for age and sex in a particular environment.

POPULATION SURVEYS

A precise assessment of the "normal"Hb concentration is of more than just academic interest because of the increasing interest in community health, and in particular in the prevalence of anaemia in various population groups.

In determining the "normal" Hb concentration of a population group it is essential to ensure the good health of the subjects studied, for the significance of the results obtained depends on the extent of the screening criteria used. Because of its relative frequency, it is important to exclude iron deficiency, for it is well recognised that the early stages of this deficiency may be associated with a fall in the Hb concentration of the particular subject without overt haematological changes (Bothwell and Finch, 1962; Bainton and Finch, 1964), and on the other hand patients with overt iron deficiency anaemia may escape detection for they often have no definite symptoms (Elwood, 1968; Elwood, Waters, Greene, Sweetnam and Wood, 1969). One way to exclude significant iron deficiency in apparently healthy men and women is to examine the bone-marrow iron stores. Using this method, Scott and Pritchard (1967) and Hallberg, Hallgren, Hollender, Hogdahl and Tibblin (1968) found the lowest Hb concentration at which iron was consistently present in the bone-marrow of non-pregnant women was 12·0g/100 ml. Recent studies in Sweden (Garby, Irnell and Werner, 1967) and Norway (Natvig and Vellar, 1967; Natvig, Vellar and Andersen, 1967) have drawn attention to the value of therapeutic iron trials in apparently healthy individuals in determining "optimal" values for the various haematological indices of anaemia.

Table 1 summarises the proposed normal Hb values for males and females at different ages in the Norwegian survey. These results are in general agreement with the Hb values found in previous surveys of unselected healthy subjects in Britain (Berry, Cowin and Magee, 1952) and Australia (Walsh, Arnold, Lancaster, Coote and Cotter, 1953; Davis, Kelsall, Stenhouse, Woodliff and Wearne, 1969). However, in these latter surveys, although the Hb values for men were essentially the same, the range of values for women* were lower than in the Norwegian survey, which may reflect the inclusion of some women with latent iron deficiency who were especially excluded from the Norwegian survey. Natvig and co-workers (Natvig and Vellar, 1967 / Natvig et al, 1967) applied their criteria to determine the incidence of anaemia in apparently healthy Norwegian subjects (Table 2). In children (both boys and girls) anaemia was most prevalent around puberty and early adolescence (10 - 16 years). In adult men sub-normal Hb values (below 14g%) were most frequently found after the age of 40. In women, however, sub-normal Hb values (below 12·5g%) were common in all age groups, the incidence reaching a peak between 30 and 40 years, but remaining relatively high thereafter. It is of interest to compare the results of the Norwegian survey conducted in affluent peace-time with the M.R.C. survey

*Footnote:

Berry et al (1952)	14·0±1·25 g%	(11·5 = M-2S.D)
Walsh et al (1953)	13·9±1·16 g%	(11·6 = M-2S.D)
Davis et al (1969)	14·3±1·2 g%	(11·9 = M-2S.D)
Natvig and Vellar (1967)	14.3±0.9 g%	(12·5 = M-2S.D)

of Hb levels in Britain during World War II (Medical Research Council, 1945). There was a relatively high incidence of anaemia among civilian men and women (Table 3), and children (Table 4), much higher than in the above Norwegian survey (Table 2). There was also a striking difference in the incidence of anaemia between civilian men and Canadian soldiers stationed in Britain (Table 3). This in itself suggested that the civilian Hb values were sub-optimal, and may reflect the more rigid medical criteria for selection of the soldiers and their better nutritional background. Furthermore, Berry et al (1952) obtained a higher mean Hb value of 15·7g/100 ml on 245 men tested in Britain some years after the war, compared with the M.R.C. survey mean value of 15·1g/100 ml. These differences in Hb values emphasise the importance of rigid criteria of good health and the exclusion of iron deficiency and other factors which may affect the Hb concentration when selecting a group of subjects for determining the optimal range of Hb values.

On the basis of these and similar surveys throughout the world (Table 5; Bureau of Nutrition Surveys, 1945), the W.H.O. (1968) have proposed certain criteria for the diagnosis of anaemia which are summarised in Table 6. However, some workers may prefer to use their own or other criteria, but whatever criteria are adopted, the results of Hb surveys

should be reported not only as the mean ± S.D., but also as a percentage incidence in 1 g% (or smaller) Hb increments, so that the data can be analysed by different criteria of anaemia should the necessity arise.

CLINICAL SIGNIFICANCE OF SUB-OPTIMAL Hb LEVELS

While the concept of a "normal" range of Hb values is useful for population surveys and clinical screening, it overlooks the problem of the individual patient whose Hb concentration, although within the "normal" range, is below his optimal value. This applies particularly to the early stages of iron, B_{12} and folate deficiencies, which are of particular nutritional significance in population surveys.

Iron deficiency. The well documented sequential changes in the development of iron deficiency are shown in Fig. 1. From this it can be seen that the iron stores are depleted by the time the Hb starts to fall. However, at this stage the Hb level, although sub-optimal for the patient, may be still within the normal range and an examination of the blood film may not reveal any significant morphological changes. In a study of apparently healthy adults, Natvig and Vellar (1967) found a higher incidence of subnormal MCHC values than of subnormal Hb values which led them to suggest that a subnormal MCHC might be a useful index of mild uncomplicated iron deficiency in cases where the Hb concentration is still within the normal range. These subjects

responded to iron therapy with a rise in the MCHC to normal and an increase in the Hb concentration, even when this was initially within the normal range (Table 7). Hallberg et al (1968), using the absence of reticular iron in the bone-marrow as an index of iron deficiency, showed that there was a considerable overlap of the frequency histograms of Hb levels of women with and without reticular iron in the marrow, but that the Hb levels of the latter group were shifted towards lower values (Fig. 2).

The optimal Hb concentration will therefore be under-estimated if subjects with latent iron deficiency are not excluded from the population sample and conversely the prevalence of iron deficiency may be under-estimated if only anaemic subjects are considered. Some epidemiologists may argue that this is of little clinical significance in relation to symptoms and physical activity (Elwood, 1968; Elwood et al, 1969, Simpson and Gourley, 1970) while others suggest that even slight iron deficiency may affect work capacity (Beutler, Larsh and Gurney, 1960; Hallberg, Hogdahl, Nilsson and Rybo, 1966). However, in nutrition surveys, where it is important to know the full extent of the problem, it is essential to have specific tests for iron deficiency, in addition to the measurement of Hb concentration and MCHC, which can be applied to large population samples. In this respect the assessment of

bone-marrow iron, although probably the best index of body iron status, is not a practical proposition. On the other hand, there is a marked overlap between normal and iron deficient subjects by the conventional tests of iron deficiency (viz serum iron, TIBC and percentage transferrin saturation) (Hallberg et al, 1968). Preliminary observations by Brozović (1970), following up an observation by Najean et al (1964), suggest that the in vitro uptake of transferrin-bound Fe^{59} by reticulocytes correlates well with bone-marrow iron stores in uncomplicated iron deficiency. However, in the hypochromic anaemia of chronic inflammatory or neoplastic disorders the in vitro reticulocyte uptake of Fe^{59} may not accurately reflect bone-marrow iron stores (Fig. 3), but correlates well with response to iron therapy (Brozović, 1970). This is a simple and rapid in vitro test which may prove useful in the interpretation of equivocal serum iron and TIBC results, and may well have a useful application in nutrition surveys.

Vitamin B_{12} deficiency. The development of sensitive and specific microbiological assays for B_{12} in serum and other tissues has made it possible to diagnose deficiency at an early stage, and it is now well recognised that a person may have severe B_{12} deficiency before the onset of anaemia (Fig. 4). However, careful examination of the blood film and bone-marrow

of such patients will reveal early megaloblastic changes, which are reversible by treatment with B_{12}. This treatment will also raise the Hb concentration, even when the initial Hb level is within the normal range.

Most of our knowledge of the patho-physiological effects of B_{12} deficiency in man has been derived from the study of pernicious anaemia. The non-anaemic stage of pernicious anaemia may be asymptomatic - "latent" pernicious anaemia (Mollin, 1959; Witts, 1960; Callender and Spray, 1962; Beveridge, Bannerman, Evanson and Witts, 1965), but such cases are rarely detected in clinical practice except by chance or by deliberate screening for B_{12} deficiency. However, patients with severe B_{12} deficiency may suffer serious neurological damage even in the absence of anaemia. As a group, patients with subacute combined degeneration of the spinal cord (SACD) tend to have higher Hb levels than patients with uncomplicated overt pernicious anaemia (Fig. 5), although the serum B_{12} levels are as low as in severe pernicious anaemia (Fig. 6). Patients may also present during the non-anaemic stage of B_{12} deficiency with a wide range of less specific symptoms including psychosis, glossitis, paraesthesiae, infertility and such vague complaints as lethargy, apathy, anorexia (Waters and Mollin, 1970a).

Although B_{12} deficiency may cause a wide range of symptoms before the onset of anaemia, the pathogenesis of anaemia in severe B12 deficiency is still uncertain. As already pointed out, there is no correlation between the serum B_{12} concentration and the Hb concentration. On the other hand, there is a progressive fall in the serum folate level with increasing anaemia in patients with pernicious anaemia, which is independent of the serum B_{12} level (Fig. 7) (Waters and Mollin, 1963). It is therefore tempting to suggest that folate deficiency precipitates the onset of anaemia in pernicious anaemia. However, very few patients with pernicious anaemia have serum folate levels in the range found in overt folate deficiency (Waters and Mollin, 1963). Nevertheless, owing to the postulated "B_{12} block" in the utilisation of folate in pernicious anaemia (Waters and Mollin, 1961; 1963; Herbert and Zalusky, 1962; Buchanan, 1964), it is possible that signs of folate deficiency might appear at a higher serum folate level than in the absence of B_{12} deficiency. Thus, in a particular patient, the onset of megaloblastic anaemia would depend not only on the serum B_{12} level, but also on the serum folate level necessary to overcome the "B_{12} block" (Waters, 1963). Whatever the explanation, it is significant that in patients with severe B_{12} deficiency, a surfeit of folate in the diet, whether naturally as in vegans (Wokes, Badenoch and Sinclair, 1955; Wokes and Smith, 1962; Waters, 1963), or by prophylactic supplementation with folic acid (Conley and Krevans, 1951; de Wit, 1952; Victor and Lear, 1956;

Crosby, 1960; Challenor and Korst, 1960; Ellison, 1960; Baldwin and Dalessio, 1961) will tend to delay the haematological manifestations of B_{12} deficiency while allowing neurological and other complications to develop. In fact, amongst patients with pernicious anaemia, those with SACD tend to have the highest Hb and serum folate concentrations and the lowest serum B_{12} concentrations (Mollin, 1959; Waters and Mollin, 1963; 1970b).

Folate deficiency. As in iron and B_{12} deficiency, significant folate deficiency may occur while the Hb concentration is still within the normal range. Furthermore, at this early stage of deficiency, morphological changes in the blood and bone-marrow are minimal and may be overlooked, especially when large numbers of films have to be examined, as in a busy diagnostic laboratory or in population surveys. Consequently, the presence of early folate deficiency may be missed unless specific diagnostic tests are carried out.

Herbert (1962) has documented the sequence of biochemical and haematological changes in the development of folate deficiency in a healthy adult whose dietary folate intake was reduced to approximately 5μg per day of L. casei-active folate (Fig. 8). A fall in the serum folate concentration (after only 3 weeks) was the first indication of developing folate deficiency, followed much later (after 17 weeks) by a fall in the red cell folate concentration. The first haematological abnormality to appear was nuclear hypersegmentation

of the neutrophil leucocytes as indicated by an increase in the mean lobe count (after 7 weeks). However, definite macrocytosis appeared much later (after 18 weeks) followed by megaloblastic change in the bone-marrow (after 19 weeks) and last of all by anaemia. Similar observations have been reported by Mollin and Hoffbrand (1965), who compared these various parameters of folate deficiency in a number of patients suffering from a wide variety of haematological and other conditions.

The red cell folate concentration is particularly useful in assessing the severity of folate deficiency in the absence of megaloblastic anaemia in a patient who nevertheless has a subnormal serum folate concentration. In this respect it is probably the best single index of significant folate deficiency when assessing the incidence of deficiency of this vitamin in population surveys.

Table 8 summarises some recent findings in relation to the foregoing from a recent survey of health old people (65 years and over) organised by the United Kingdom Ministry of Health and Social Security (to be published). These results show that there was a significant deficiency of iron, B_{12} and folate in subjects whose Hb levels were within the accepted normal range, as well as in the anaemic subjects. On the other hand, there was no biochemical evidence of deficiency of these nutrients in a large proportion of subjects with subnormal Hb levels. This raises the possibility that the arbitrary lower normal Hb level may have been set too high for old people (viz, males 13 g%, females 12 g%), and similar observations

have been reported by Walsh et al (1953), Vellar (1967), Kaufman, Grant and Moorhouse (1969) and Weatherburn et al (1970). The explanation for these lower Hb levels in old people is uncertain, and the final report of the recent Ministry of Health survey may help to elucidate this. Apart from possible nutritional differences, other factors peculiar to this age group may be important in suppressing haemopoiesis, and in this respect altered hormonal balance and diminished physical activity may be significant and require investigation.

In Table 8 iron deficiency was assessed in terms of a transferrin saturation less than 16%, which probably over-estimates the extent of true iron deficiency. Using a combination of parameters (viz, serum iron <65μg% and TIBC >410μg%), the incidence of iron deficiency in the same population was approximately 9% (Brozović, unpublished data), compared to 20% based on transferrin saturation alone. There was a surprisingly low incidence (2·7%) of significant folate deficiency, as determined by a red cell folate concentration less than 100 ng/ml, but a much higher incidence (14%) of mild or incipient folate deficiency as indicated by a low serum folate concentration (<3ng/ml). The incidence of approximately 1% of serum B_{12} concentrations less than 100 pg/ml (i e in the range found in overt pernicious anaemia) is ten times that of "clinical" pernicious anaemia in Britain reported by Scott (1960). The survey also revealed a 10% incidence of serum B_{12} concentrations in the borderline range (100-200 pg/ml).

The clinical significance of this borderline range is uncertain, but such levels occur in early pernicious anaemia (Mollin, 1959; Callender and Spray, 1962), atrophic gastritis (Whiteside, Mollin, Coghill, Williams and Anderson, 1964) and in many patients following partial gastrectomy (Deller and Witts, 1962; Hines, Hoffbrand and Mollin, 1967) and probably indicate mild B_{12} deficiency. The survey has therefore revealed a much higher incidence of potential B_{12} deficiency in old people than previously suspected.

PHYSIOLOGICAL ADAPTATION TO SUB-OPTIMAL Hb LEVELS

Although deficiencies of iron, B_{12} and folate may produce definite symptoms in their own right, it is still an open question whether mild anaemia per se results in any functional impairment or lack of well-being owing to the existence of adaptive mechanisms which compensate for the decrease in arterial blood O_2 content due to anaemia.

Perhaps the most important of these mechanisms is the decreased affinity of Hb for O_2 in anaemia (Fig. 9), which is mediated by an increase in red cell 2, 3-diphosphoglycerate (2, 3-DPG), an intermediate metabolite in the red cell glycolytic (Emden-Meyerhoff) pathway (Chanutin and Curnish, 1967; Benesch and Benesch, 1969). In vivo studies have shown that there is an increase in red cell 2, 3-DPG at high altitude (Lenfant, Torrance, English, Finch, Reynafarje, Ramos and Faura, 1968; Eaton, Brewer and

Grover, 1969; Torrance, Jacobs, Restropo, Eschbach, Lenfant and Finch, 1970), in cardiac failure (Woodson, Torrance and Lenfant, 1969) and in anaemia (Torrance et al, 1970). The linear relationship between 2, 3-DPG and Hb concentration demonstrated in anaemia and at high altitudes also exists in healthy subjects with normal Hb levels (Fig. 10) (Eaton and Brewer, 1968; Torrance et al, 1970). The increase in 2, 3-DPG is also associated with an increase in ATP, but the overall effect of 2, 3-DPG on Hb-O_2 dissociation is about nine times that of ATP (Torrance et al, 1970).

Changes in pH may also affect the Hb-O_2 dissociation curve (the Bohr effect), acidosis leading to a right shift and alkalosis a left shift. In a recent study of anaemic patients, Torrance et al (1970) showed that acidosis was associated with a smaller increment in 2, 3-DPG than expected from the Hb concentration, and vice versa for alkalosis (Fig. 11). The relative effect of the pH and DPG adaptive mechanisms was studied in subjects exposed to sudden hypoxia of altitude (Lenfant et al, 1968). These workers showed that when normal subjects arrived at high altitude there was a rapid increase in 2, 3-DPG ($t_{\frac{1}{2}}$ approximately 6 hours), which reached a maximum by 24 hours. At the same time there was a marked alkalosis due to hyperventilation, which shifts the in vivo Hb-O_2 dissociation curve to the left, thus aggravating the anoxia already present, and possibly enhancing the rapid formation of DPG which then shifts the curve back to the right.

The DPG mechanism is therefore of fundamental importance in the rapid metabolic adaptation to tissue anoxia, whether this is due to anaemia or to cardio-pulmonary causes. In mild to moderate anaemia this mechanism may maintain adequate tissue oxygenation without affecting cardio-pulmonary function, and thus account for the minimal symptomatology of such patients. The precise relationship between this mechanism and erythropoietin stimulation is uncertain, but probably the erythropoietin mechanism is stimulated when the DPG mechanism is unable to keep pace with tissue O_2 demand.

SUMMARY AND CONCLUSIONS

The differentiation between anaemia and normality based on an arbitrary Hb concentration remains a statistical concept. The results of random surveys of Hb concentrations, although representative of the group under study, may not represent optimal levels. This shortcoming may be largely of academic interest when the main objective is the detection and treatment of overt anaemia in under-developed countries where nutritional anaemia is a serious public health problem. On the other hand, in clinical medicine the individual patient's optimal Hb concentration is the significant parameter and the acceptance as normal of a Hb concentration within the statistical "normal" range may result in delayed diagnosis of latent deficiencies of iron, B_{12} or folate, which may reduce the Hb concentration below its optimal level in apparently healthy subjects. However, careful

screening of the blood film and specific tests for iron, B_{12} and folate deficiences, especially in patients and population groups at risk, will greatly help in the early detection of these deficiencies before the onset of overt anaemia or other complications.

To make this a feasible proposition for clinical screening and nutritional surveys it will be necessary to automate the measurement of as many nutritional parameters as possible. Progress has been made in the automation of methods for the measurement of serum iron and TIBC using auto-analysers (Zak and Epstein, 1964; Young and Hicks, 1965; Giovanniello, di Benedetto, Palmer and Peters, 1968; Brozovič and Copestake, 1969). The microbiological assay of folate in serum and whole blood has also been successfully automated (Millbank, Davis, Rawlins and Waters, 1970). This was made possible by the use of a chloramphenicol-resistant strain of the assay organism, Lactobacillus casei (Davis, Nicol and Kelly, 1970), which obviated the need for aseptic conditions. This method is a simplification of the standard manual methods (Herbert, 1961; Waters and Mollin, 1961) and has the capacity for dealing with 80 - 100 specimens in duplicate per hour, compared with approximately 200 specimens per week by the manual methods. This tremendous work capacity makes it especially suitable for providing a regional diagnostic service and for nutritional surveys of large population groups. The same principles are now being applied to the assay of B_{12} in serum (Millbank, Rawlins, Anderson and Waters, to be published).

The establishment of normal values for Hb concentration and various nutrients implies the comparability of results between different laboratories and necessitates standardisation of the methods used. The International Committee for Standardisation in Haematology (1967) have made recommendations for the measurement of Hb using a cyanmethaemoglobin solution as a standard, and WHO have established central reference laboratories to standardise the assays of iron, vitamin B_{12} and folate (WHO, 1968). The function of these laboratories is to distribute standards, to correlate the results of the participating laboratories and to advise on the possible cause of discrepancies where these arise.

Correlation between Hb concentration and functional capacity is still an open question, although evidence is available that physiological adaptation to sub-optimal Hb levels can maintain tissue oxygen supply without significant cardio-pulmonary embarrassment. Furthermore, with the Hb concentration, as with other physiological parameters, conditions other than nutrition may affect the level of normality at different ages and under different environmental conditions.

From these observations it is apparent that, while it is still essential to define anaemia as a parameter of health, the Hb concentration cannot be regarded as a sensitive index of nutritional status. This is better determined by direct measurements of the nutrients concerned, and where possible by careful examination of the blood film, especially in patients and population groups at risk.

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TABLE I

NORWEGIAN SURVEY OF HAEMOGLOBIN CONCENTRATIONS [1]

Proposed normal values and criteria for anaemia

(Natvig et al, 1967; Natvig and Vellar, 1967)

Age (Years)	Male	Anaemia (Hb below)	Female (Non-pregnant)	Anaemia (Hb below)
7 - 9	12·7±1·6[2]	11·0	12·7±1·6*	11·0
10 - 13	13·2±1·6	11·5	13·2±1·6	11·5
14 - 16	15·0±2·0	13·0	14·2±2·0	12·0
17 - 20	15·5±2·0	13·5	14·2±2·0	12·0
>20	15·7±1·8	14·0	14·3±1·8	12·5

(1) g. per 100 ml
(2) Mean ±2SD (=95% of population)

TABLE 2

INCIDENCE OF ANAEMIA IN CONTROL SUBJECTS

(Natvig and Vellar, 1967; Natvig et al, 1967)

	MALE		FEMALE	
Age (Years)	Hb below (g%)	% Anaemic	Hb below (g%)	% Anaemic
7 - 9	11.0	0.6	11.0	0.6
10 - 13	11.5	2.1	11.5	2.1
14 - 16	13.0	2.9	12.0	3.4
17 - 20	13.5	0.7	12.0	1.7
20 - 29	14.0	1.9	12.5	3.4
30 - 39	14.0	1.1	12.5	12.5
40 - 49	14.0	4.8	12.5	3.9
50 - 59	14.0	4.1	12.5	6.8
60+	14.0	6.2	12.5	7.1

TABLE 3

M.R.C. SURVEY OF Hb LEVELS IN GREAT BRITAIN IN 1943 (1)

Incidence of anaemia (2) in civilian men and women and

Canadian soliders stationed in Great Britain

	Civilians (3)			Canadian soldiers (4)
	Single Women	Married Women	Men	
Below 12.0 (80%)	5.8%	9.6%		
Below 12.5 (85%)	14.9%	19.9%		
Below 13.0 (90%)			7.4%	0.2%
Below 14.0 (95%)			17.5%	0.5%

(1) M.R.C. Special Report Series No. 252 (1945)

(2) Using criteria for anaemia in Table 1 viz:-

Men Hb <14.0g/100 ml (≙ 95% Haldane)
Women Hb <12.5g/100 ml (≙ 85% Haldane)

(3) From Table VI, p25, MRC Report (1945)

(4) From Table XXXIV, p106, MRC Report (1945)

TABLE 4

M. R. C. SURVEY OF Hb LEVELS IN GREAT BRITAIN IN 1943 (1)

Incidence of anaemia in children

Age (Years)	Anaemia (2) (Hb less than)	Incidence (%) (3)	
		Boys	Girls
5 - 10	<75%	3·9% (0·6) (4)	3·8% (0·6) (4)
11 - 14	<80%	4·4% (2·1)	9·0% (2·1)
15 - 19	<90%	8·1% (2·9)	26·5% (3·4)

(1) MRC Special Report Series No. 252 (1945)

(2) Approximate equivalents to criteria for anaemia in Table 1 expressed as Hb% (Haldane)

(3) From Table XIV, p32, MRC Report (1945)

(4) Figures in brackets indicate corresponding incidence of anaemia in the Norwegian survey (see Table 2)

TABLE 5

PUBLISHED CRITERIA FOR ANAEMIA IN ADULT MEN AND WOMEN (1)

Author	Country	Men	Women
Wintrobe (1933)	U.S.A.	14.0	12.0
Bierring (1940)	Denmark	13.0	11.5
Berry et al (1952)	Britain	13.5	11.5
Walsh et al (1953)	Australia	13.5	11.6
Kilpatrick (1961)	Britain	12.5	12.0
Scott and Pritchard (1967)	U.S.A.	...	12.0
Garby et al (1967)	Sweden	...	11.5
Natvig and Vellar (1967)	Norway	14.0	12.5
Hallberg et al (1968)	Sweden	13.0	12.0
W.H.O. (1968)	Composite series	13.0	12.5
Dacie and Lewis (1968)	Composite series	13.5	11.5
Davis et al (1969)	Australia	13.3	11.9
Weatherburn et al (1970)	Canada	12.5	10.8

(1) Hb g% below which anaemia occurs - Mean - 2 S.D.

TABLE 6

WHO CRITERIA FOR THE DIAGNOSIS OF ANAEMIA (1968)

	Hb g/100 ml
Children:	
6 months to 6 years	11 (1)
6 years to 14 years	12
Adult females:	
non-pregnant	12
pregnant	11
Adult males	13

(1) >95% of normal subjects have Hb concentrations higher than the values given. However, upward correction must be made for high altitudes.

TABLE 7

EFFECT OF IRON THERAPY ON THE Hb AND MCHC OF 25 MEN AND 23 WOMEN WHOSE INITIAL MCHC WAS SUBNORMAL (i.e. <30.5%) (1)

Duration of iron therapy	Men (2)		Women (3)	
	Hb (g%)	MCHC (%)	Hb (g%)	MCHC (%)
Before iron	14.54	29.6	12.88	29.0
After 1 month	15.42	33.8	13.80	32.7
After 2 months	15.37	33.5	13.78	32.2
After 3 months	15.67	34.0	14.25	33.6
Normal	15.7	33.6	14.3	33.6

(1) From Natvig and Vellar (1967)

(2) Only 3 of the 25 men had Hb <14g%

(3) Only 8 of the 23 women had Hb <12.5g%

TABLE 8

INCIDENCE OF IRON, B_{12} AND FOLATE DEFICIENCY IN HEALTHY GERIATRIC SUBJECTS (≥65 YEARS) IN RELATION TO Hb CONCENTRATION

Hb concentration	No. studied	Iron deficiency (Transferrin Saturation <16%)	B_{12} deficiency (Serum B_{12} <100 pg/ml)	Folate deficiency (RCF <100ng/ml)
Normal (1)	616	17·4%	0·8%	2·6%
Subnormal	52 (7·6%)	53·0%	2·0%	4·0%
Total	667	20·0%	0·9%	2·7%

(1) Male Hb >13g%

Female Hb >12g%

LEGENDS

Fig. 1 Sequential changes (from left to right) in the development of iron deficiency. From Simon, Giblett and Finch (1966). Reproduced by kind permission of University Press, Seattle.

Fig. 2 Comparison of the frequency histograms of haemoglobin concentrations of 193 women who had stainable iron in the bone-marrow (continuous line) and 67 women who lacked such iron. From Hallberg et al (1968). Reproduced by kind permission of Almqvist and Wiksells, Uppsala.

Fig. 3 Relationship between the in vitro uptake of ^{59}Fe by reticulocytes and stainable iron in the bone-marrow in 33 anaemic patients (Hb 5·0 - 10·5g%) suffering from conditions known to be associated with iron deficiency.

The stippled area indicates the normal range of ^{59}Fe uptake by reticulocytes. Normal amounts of iron in the bone-marrow are indicated by Grades 2 and 3; reduced iron by Grades 0 and 1; increased iron by Grade 4. Circles represent results in males; triangles females. Closed symbols indicate normal and open symbols increased ^{59}Fe uptake by reticulocytes. From Brozović (1970)

Fig. 4 Relationship between the serum B_{12} and haemoglobin concentrations (100% = 14·8g/100 ml) of 92 patients with pernicious anaemia. The starred circles indicate patients with subacute combined degeneration of the cord. From Anderson (1965).

Fig. 5 Haemoglobin concentrations (g/100 ml) of 17 patients with subacute combined degeneration of the cord (closed circles) compared with the mean haemoglobin concentrations ($\pm$ 1 S.D.) of 59 patients suffering from uncomplicated pernicious anaemia. From Waters and Mollin (1970b). Reproduced by kind permission of the Wellcome Trust.

Fig. 6 The serum B_{12} concentrations in (1) a group of patients with early pernicious anaemia who were not anaemic and not complaining of symptoms of B_{12} deficiency, (2) a group of patients with subacute combined degeneration of the cord without anaemia, and (3) a group of non-anaemic patients with pernicious anaemia whose only symptom was glossitis. From Mollin (1959). Reproduced by kind permission of Athlone Press, London.

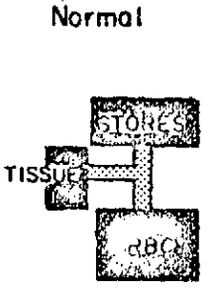
Fig. 7 Serum folate (L. casei) concentrations of 119 patients with pernicious anaemia in relation to the degree of anaemia. Open circles indicate patients who presented with subacute combined degeneration of the cord. The continuous oblique line joins the mean serum folate concentration in each group; the differences between the means were highly significant ($P<0.001$). The serum B_{12} concentration (range and mean) for each group is indicated at the top of each column. The broken horizontal lines at 6 and 18 mμg/ml (ng/ml) indicate the range of serum folate concentrations found in 95% of normal subjects. From Waters (1963).

Fig. 8 Sequence of biochemical and haematological changes in the development of folate deficiency due to sudden reduction of dietary folate in a previously healthy adult male. From Herbert (1962). Reproduced by kind permission of Trans. Ass. Amer. Phycns.

Fig. 9 Average change of P_{50} (pH 7.4) (i e P_{O_2} in m m Hg required for 50% saturation of blood with oxygen at pH 7.4) as a function of haemoglobin concentration. From Lenfant et al (1969). Reproduced by kind permission of Plenum Press, New York.

Fig. 10 Average change of intra-erythrocyte DPG (2, 3-diphosphoglycerate) as a function of haemoglobin concentration. From Lenfant et al (1969). Reproduced by kind permission of Plenum Press, New York.

Fig. 11 Average deviation of measured DPG from calculated DPG (using the regression line DPG = -0·194 Hb + 8.89) as a function of arterial pH. These results from Lenfant et al (1969) were based on data from a group of anaemic patients at Medellin (Columbia). Reproduced by kind permission of Plenum Press, New York.



	Normal	Latent Iron Deficiency	Early Iron Deficiency Anemia	Late Iron Deficiency Anemia	Tissue Iron Deficiency
R-E Marrow Iron →	Normal	Reduced	Absent	Absent	Absent
Transferrin Saturation →	30-45%	30-45%	<15%	<10%	<10%
Anemia →	Absent	Absent	Normocytic normochromic	Microcytic hypochromic	Microcytic hypochromic
Sideroblasts →	40-60%	40-60%	<10%	<5%	
					Spoon nails Glossitis Dysphagia

Figure 1

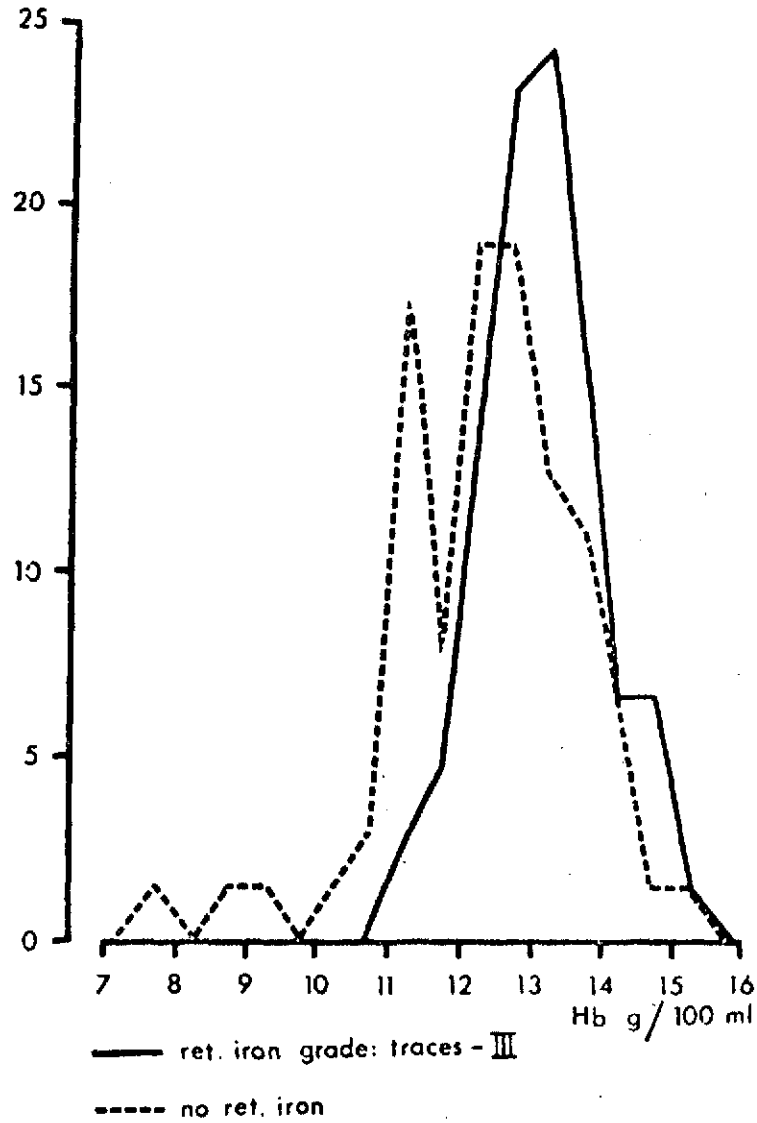

25
20
15
10
5
0
7
8
9
10
11
12
13
14
15
16
Hb g/100 ml
ret. iron grade: traces - III
no ret. iron

Figure 2

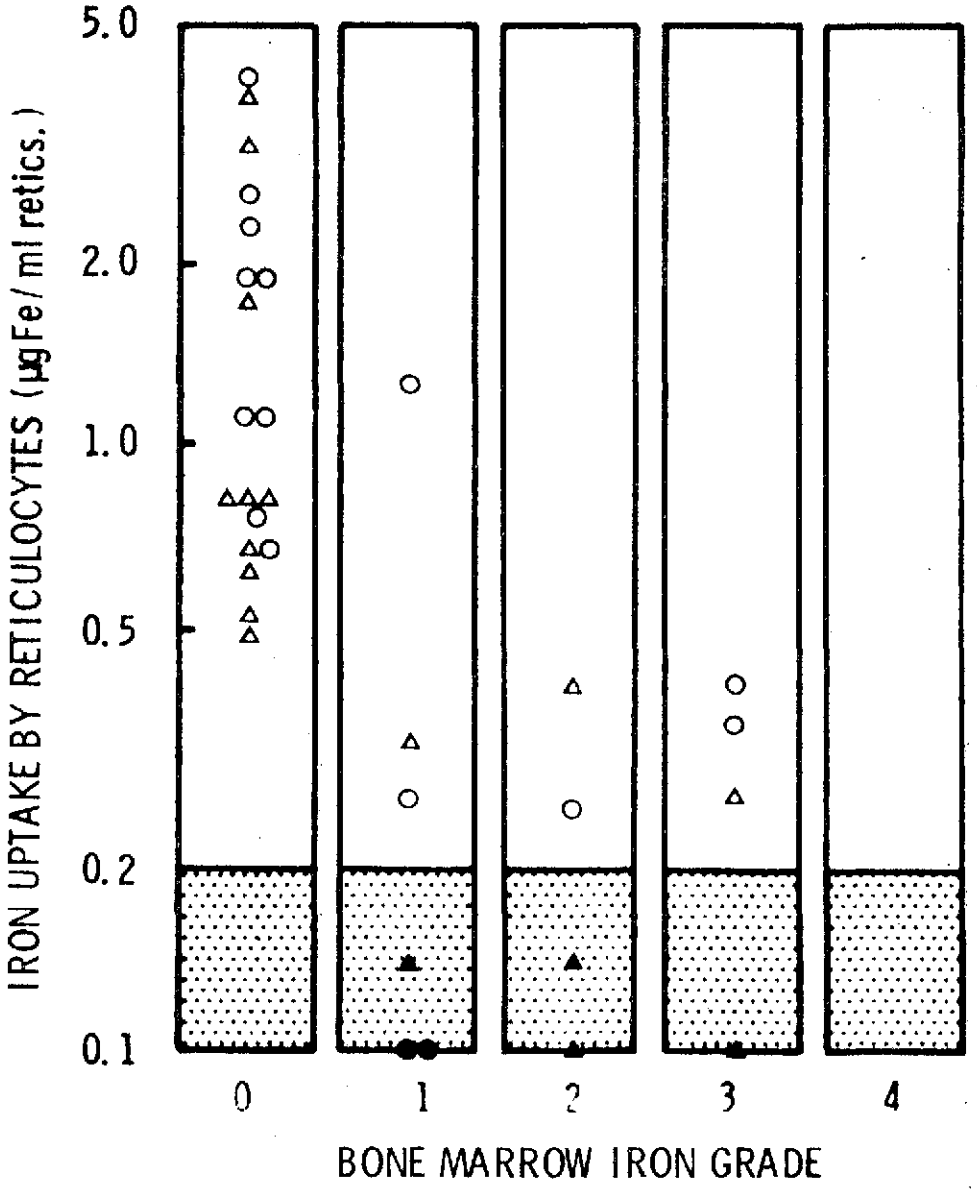

5.0
2.0
1.0
0.5
0.2
0.1
IRON UPTAKE BY RETICULOCYTES (µgFe/ml retics.)
0
1
2
3
4
BONE MARROW IRON GRADE
BROZOVIC. (1970)

Figure 3

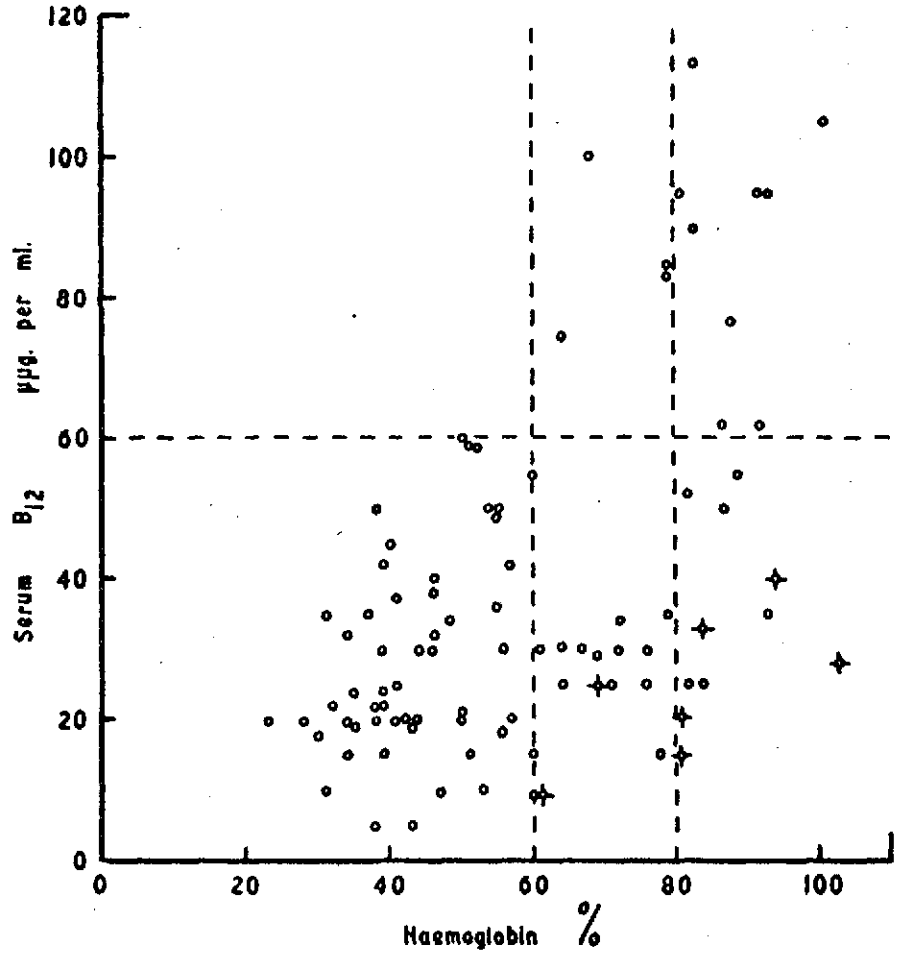

120
100
80
60
40
20
0
Serum B_{12} µµg. per ml.
0
20
40
60
80
100
Haemoglobin %

Figure 4

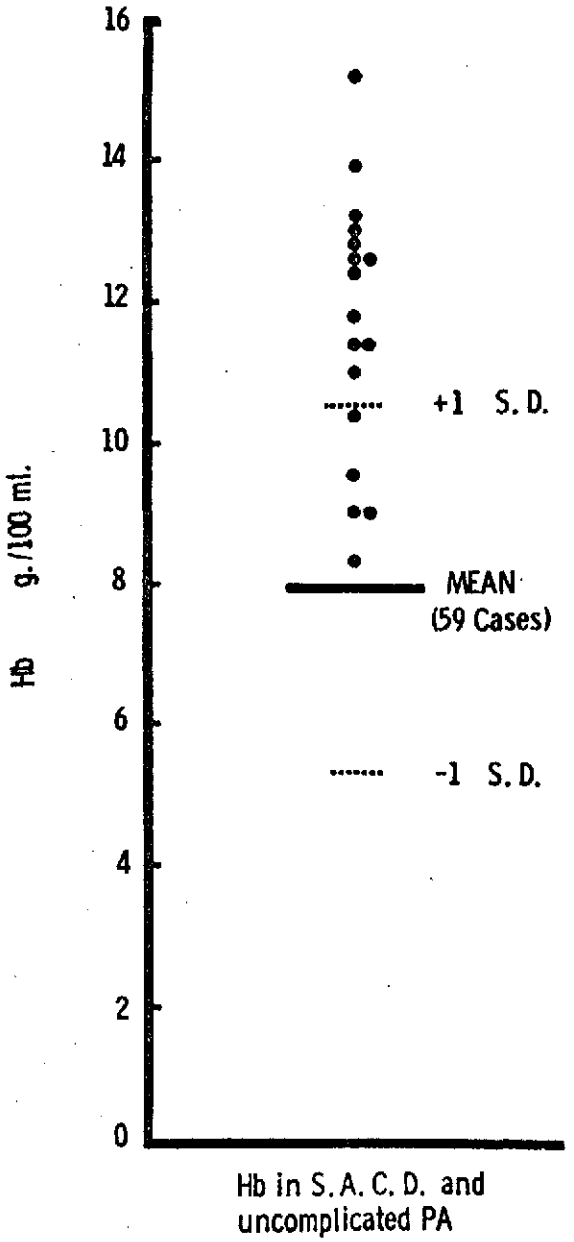

16
14
12
10
8
6
4
2
0
Hb g./100 ml.
+1 S. D.
MEAN
(59 Cases)
-1 S. D.
Hb in S. A. C. D. and
uncomplicated PA

Figure 5

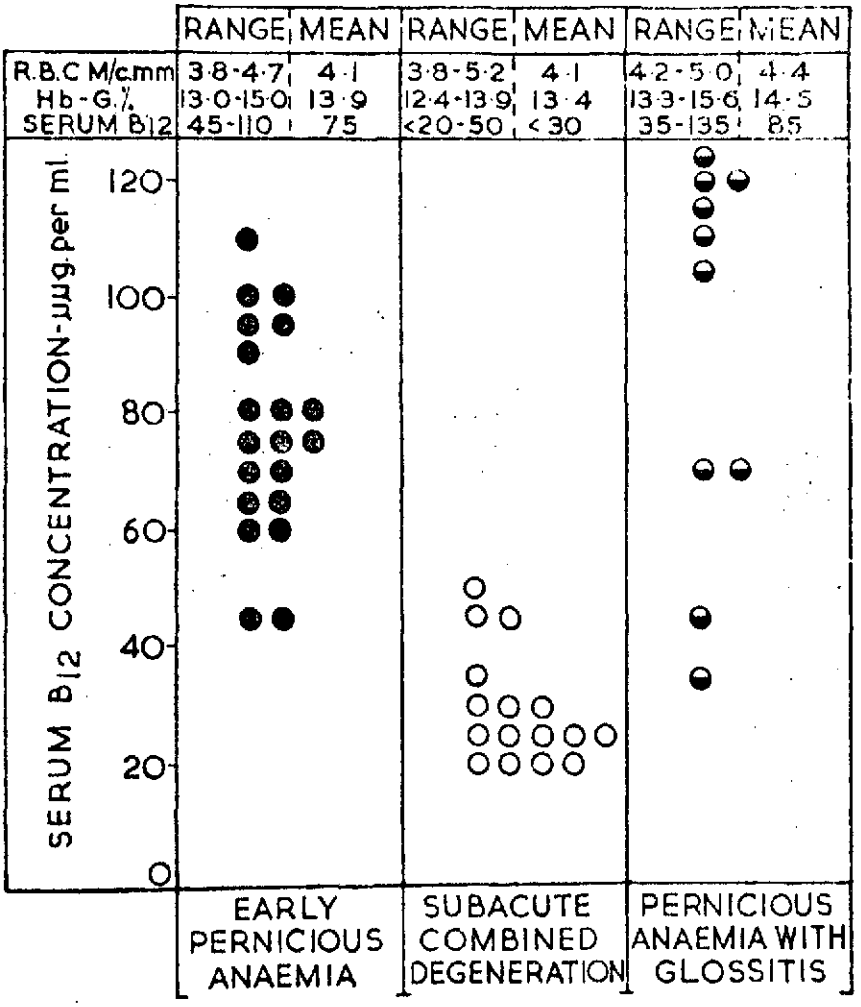

RANGE
MEAN
RANGE
MEAN
RANGE
MEAN
R.B.C M/c.mm
3·8-4·7
4·1
3·8-5·2
4·1
4·2-5·0
4·4
Hb-G.%
13·0-15·0
13·9
12·4-13·9
13·4
13·3-15·6
14·5
SERUM B12
45-110
75
<20-50
< 30
35-135
85
SERUM B12 CONCENTRATION-µµg. per ml.
120
100
80
60
40
20
0
EARLY PERNICIOUS ANAEMIA
SUBACUTE COMBINED DEGENERATION
PERNICIOUS ANAEMIA WITH GLOSSITIS

Figure 6

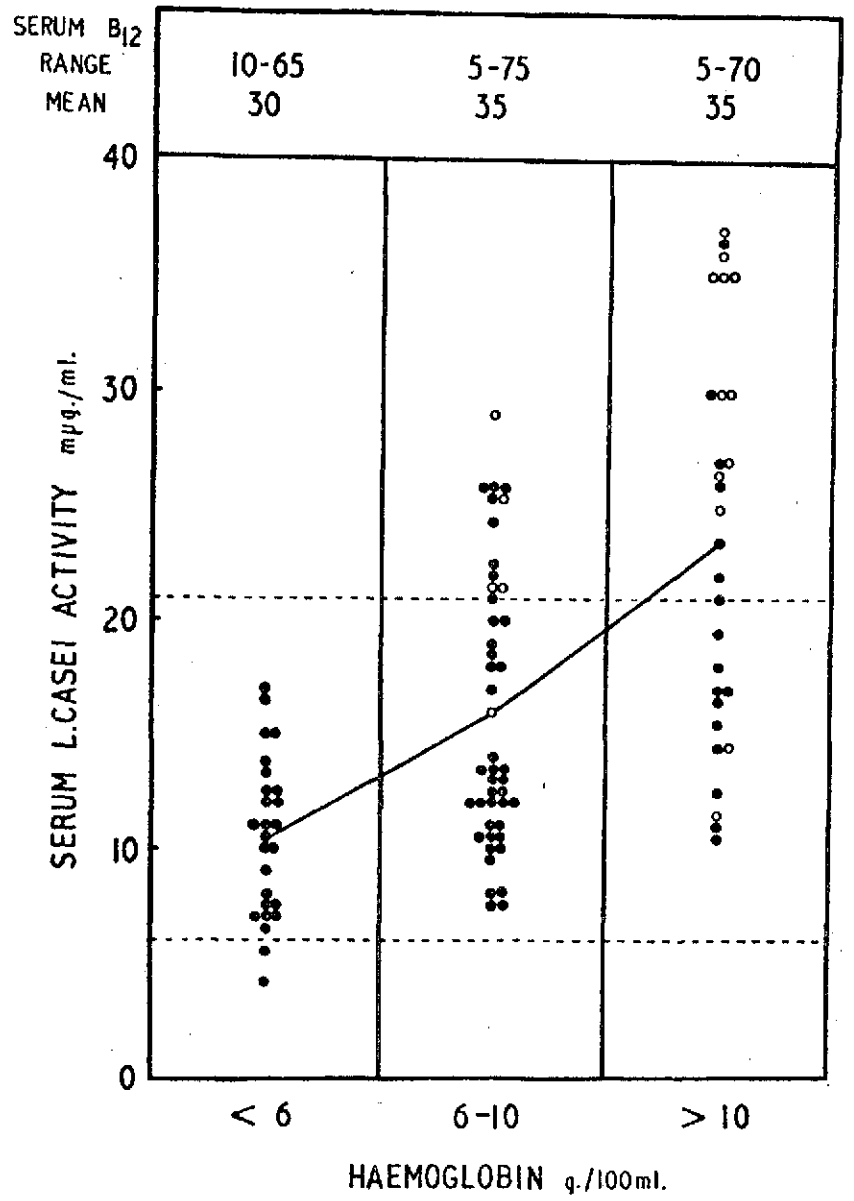

SERUM B12
RANGE
MEAN
10-65
30
5-75
35
5-70
35
40
30
20
10
0
SERUM L.CASEI ACTIVITY mμg./ml.
< 6
6-10
> 10
HAEMOGLOBIN g./100ml.

Figure 7

DIETARY FOLIC ACID DEPRIVATION IN MAN:
BIOCHEMICAL AND HEMATOLOGIC SEQUENCE OF EVENTS

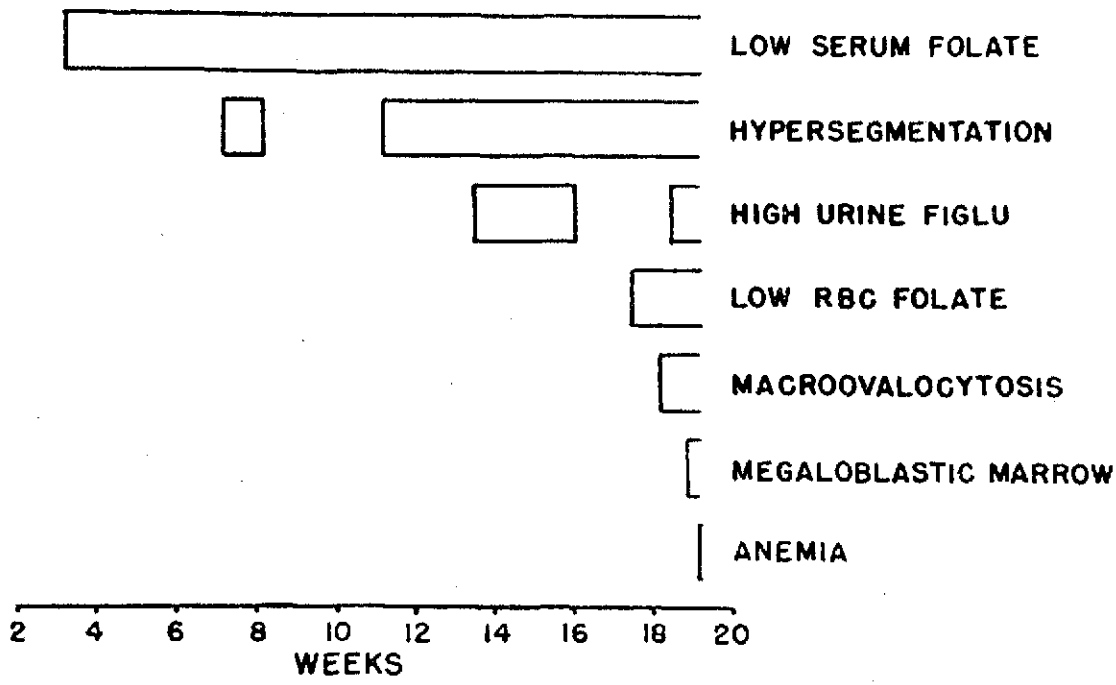


Figure 8

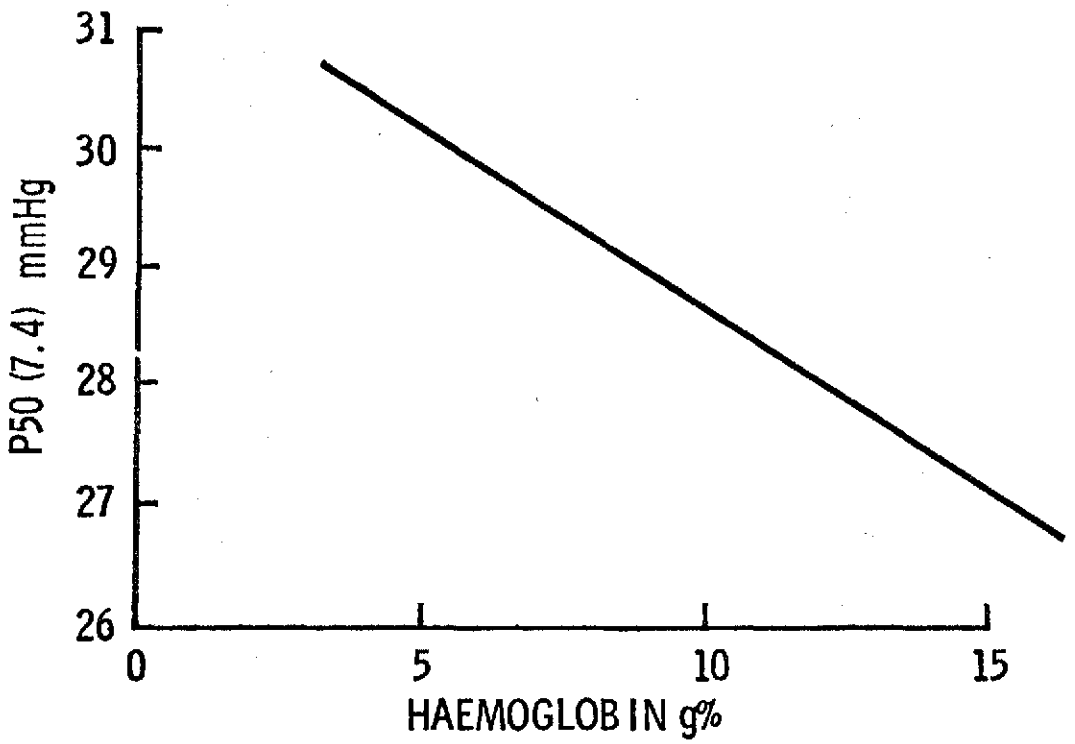


Figure 9

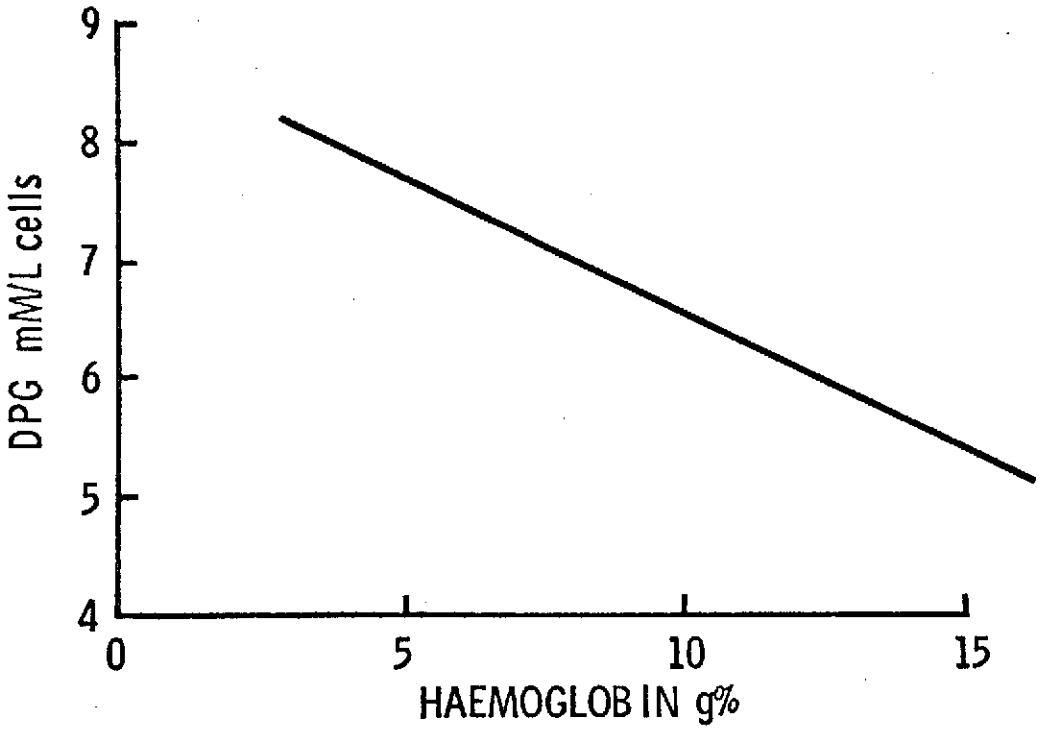


Figure 10

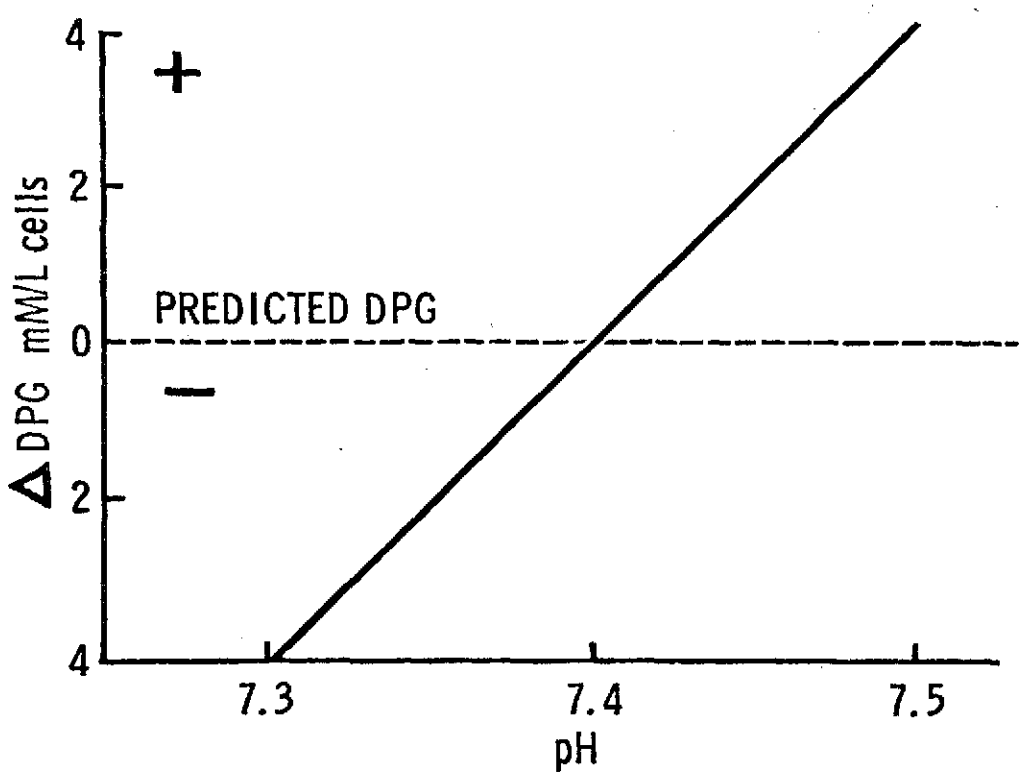


Figure 11