# BALANCE STUDIES and CLINICAL OBSERVATIONS

on

# IRON-DEFICIENCY ANAEMIA

in

# INFANCY and CHILDHOOD.

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Thesis for the Degree of M.D.,
Glasgow University,

by

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# Preface.

The subject of iron-deficiency has of recent years received great attention from clinicians and biologists. This attention is well merited because of the widespread existence of the deficiency and because of its deleterious effect upon the health of the community. Many aspects of the subject are still obscured and the present work has been undertaken to throw further light on those problems. These researches were conducted mainly in the wards and biochemical laboratory of the Royal Hospital for Sick Children, Glasgow; the work reported in Part I of this thesis was done at the Govan Welfare Centre, and I am indebted to the Managers of the Centre for rendering this investigation possible, and to Dr. Ethel Crawford for providing the clinical material.

It is a pleasure to express my indebtedness to Dr. Stanley G.Graham and Professor Noah Morris for their constant advice and encouragement. I am also indebted to Professor G.B.Fleming for placing cases in his wards at my disposal and for his encouragement throughout this investigation.

I wish to express my gratitude to Mr. William McCunn's Trustees for assisting me with a McCunn Research Scholarship during the past two years, and to the Medical Research Council who defrayed part of the expenses of this work.-

# Introduction.

Iron deficiency is of common occurrence in man, and is recognised by the existence of a hypochromic microcytic anaemia (Wintrobe, 1930 and 1934). It is now generally accepted that many of the anaemias frequently referred to as "secondary" in the older text-books, can be shown to have as their aetiological basis an inadequate intake or assimilation of iron. The conception of iron deficiency as a cause of disease although not new has only recently been established on a firm scientific basis, the credit for which rests very largely with British haematologists. Iron therapy has suffered from long periods of neglect and unjustifiable criticism, alternating with times when it was enthusiastically applied.

The earliest use of iron medicinally dates from early Grecian times (Hahn, 1937) and the treatment consisted in drinking water in which a sword had been allowed to rust. Iron and steel meant strength and power in battle, and in older pharmacopoeias iron is referred to as mars. Sydenham in 1661, and Willis in 1681 (Fowler, 1936) described the efficacy of iron therapy in chlorosis. Not until Menghini in 1746 (Halfer, 1930) discovered that this element was a characteristic constituent of the blood

could there be a logical explanation of its value. In 1831 Blaud announced the formula and dosage of a prescription of iron used successfully in the treatment of chlorosis. It is typical of the history of iron that the original dosage of Blaud's pills was reduced by certain physicians. A year after he had announced his observation, Blaud (1832) objected to the use of a dose smaller than he recommended; there can be no doubt that the present satisfactory position regarding the recognition of iron-deficiency anaemia and its effective treatment with iron has been greatly delayed by the use of too small doses.

This recent interest in the subject of iron metabolism has embraced the period of infancy and childhood, and several intensive researches have been made into the nutritional anaemia of infancy. Concerning this anaemia very diverse views have been held, both as to its aetiology and as to whether it can be differentiated into one or more clinical entities. It has been discussed and subdivided in the literature under the names of "nutritional anaemia", "alimentary anaemia", "cow's milk anaemia", "chlorotic anaemia of infants", "simple anaemia of infancy", "oligosidéremie", etc. Possibly the most accurate appellation would be "the iron-deficiency anaemia of infancy".

It would appear that as early as 1771, Sauvage reported a case of "chlorosis" in an infant, and in 1864, three further cases under twelve months of age were described by Nonat. In 1903, Hallé and Jolly described "a case of chlorosis" in a 3½ years old child, fed exclusively on milk and cereal, in which they considered iron treatment effective. Tixier (1911) although advocating iron treatment emphasised the need for treating the "cause" of the anaemia, mentioning as such, infections and disturbances of the digestive functions, so that it would seem he believed iron deficiency played a relatively small part in the production of anaemia in infancy.

Cautley (1910) wrote of "simple infantile anaemia" occurring usually in children between the ages of eleven months and three years, the essential cause of which is a primary deficiency of iron in the foetal tissues. He dismissed toxaemia as the cause of the anaemia and advocated treatment with small doses of an inorganic iron preparation together with mixed feeding and a low allowance of milk. He obtained cures in 3 to 4 months. Ashby (1912) published figures showing the iron content of the human liver at different ages, and calculated that the foetal iron store was exhausted by 7 months of age. Unfortunately, there is a gap in Ashby's findings: there are no examin-

ations on infants between 3 and 7 months old.

At this stage a different orientation was given to the subject by Czerny's paper in 1912, read before the Association Internationale de Pédiatrie in Paris. He set aside the theory of iron-deficiency as unsatisfactory because, he says, the addition of iron to the diet will not cure "alimentary anaemia", no matter whether the iron is given in organic or inorganic form. To cure it, he stated, it was necessary to reduce the milk in the diet to a minimum, replacing it by mixed feeding, and he held that the milk itself had an injurious effect. It was suggested that the injurious factor was cow's milk fat, which by producing soapy stools depleted the body of bases, and that the consequent acidosis produced anaemia. Kleinschmidt (1916) agreed with Czerny with certain modifications, and considered slight cases of "alimentary anaemia" very common. He considered that infants developing anaemia came of defective stock, as evidenced by the large proportion with congenital abnormalities, e.g. malformed ears, telangiectasis, hernias, etc. He stated that iron therapy had no effect on the anaemia, and attributed it to a toxic effect of the milk. He also instanced another type of "alimentary anaemia" - "Mehlnahrschaden" - in which oedema and anaemia developed on a one-sided cereal

diet. Hans Opitz of Berlin (Pfaundler and Schlossman, 1935) states that the important aetiological factor in "alimentary anaemia" is active injury to the blood components through the diet, regarding the cow's milk fat as an important haemolytic agent. Opitz apparently still regards iron deficiency as of little importance.

In 1920, however, Schwartz and Rosenthal in America stated that they found a common type of anaemia which responded excellently to iron therapy; unfortunately no details were given of a single case treated with iron alone. Nevertheless they considered that the theory of iron deficiency would only partly account for this anaemia, because in some cases an increase in haemoglobin was achieved by placing the child in the open air only; it is possible that this is an instance of the misinterpretation of the usual temporary rise in haemoglobin after 3 months of age (see Part I). In 1926, Bass and Denzer confirmed the efficacy of iron therapy in the hypochromic anaemias of infancy; they emphasised that much of the disfavour cast on iron therapy at that time was the result of misinterpreted analogy with the experimental haemorrhagic anaemia of animals. The extensive and careful researches of Mackay (1931 and 1933)(2) have produced the first authoritative figures for the haemo-

globin content of the blood of infants from within twenty-four hours of birth, and at monthly intervals up to the end of the first year of life. It is now generally accepted that the normal haemoglobin level at birth is about 143 per cent. That this falls to 106 per cent in the first two weeks of life, and reaches about 74 per cent at three months. Thereafter it rises gradually, reaching 86 per cent at six months and remains at this level during the remainder of the first year of life. She has shown also that nutritional anaemia is very common among the infant population of London, that this anaemia is due to iron deficiency, and that the administration of iron is an effective prophylactic as well as curative measure. These facts are now generally accepted in this country and U.S.A.

The reasons for this prevalence of anaemia in the early years of life lie partly in the constitutional peculiarities of the infant, partly in the external factors such as iron-deficiency, infections, etc., that disturb the equilibria on which the normal state of the blood depends. In this sense anaemia is not to be considered a disease, but is the result of a disturbance in normal physiological processes which may be brought about by a disease or constitutional defect on the one hand, and on the other by factors that are entirely extrinsic.

In the study of anaemia an attempt should be made (1) to recognise those factors, extrinsic or intrinsic, (2) to understand in what way they disturb the normal physiological processes, and (3) to learn how to prevent or correct them.

Those types of anaemia in which constitutional factors dominate the picture are often designated "primary". In such cases the anaemia exists as the result of some defect in the haematopoietic function, so that extrinsic factors have only a secondary influence. In the great majority of cases, however, the constitutional factors predispose to the development of the anaemia, but the extrinsic factors play the part of the precipitating cause, as in nutritional anaemia which is by far the commonest of all anaemias occurring during infancy and childhood.

It might be well at this stage to define the possible inferences to be drawn from the term "constitutional", the loose use of which has not tended to clarify our knowledge of the aetiology of the anaemias of infancy. "Constitution" may be grouped under three heads according to its various usages:-

(1) It is used to denote tendencies common to all individuals at a given age. In so far as such tendencies are the common property of all they are to be considered

physiological; but in some individuals they are more marked and last longer, and in this sense may be considered pathological. The lack of a well-established equilibrium results in the lability of function that is one of the characteristics of infancy, especially so in premature babies and others for any reason unready to assume the functions of extra-uterine life. Wide swings in haemoglobin, like wide swings in water balance or in temperature, may occur under provocation too slight to produce changes in older children and adults. Thus a characteristic of infancy is the ease with which the rate of haemolysis is increased, and blood formation may resort to an earlier embryonic stage. (Hawksley and Lightwood, 1934; Parsons, Hawksley, and Gittins, 1933). Another factor having a bearing on the incidence of anaemia is rapid gain in weight which results in intensification of any deficiency that may be present.

(2) Another sense in which the term constitution is used is that which explains why some individuals act differently from the average. This meaning is frequently encountered in the German literature as "konstitutionelle Minderwertigkeit". A conception as vague as this is not very useful and is likely to be applied too readily to anything not well understood. Such a conception must be

invoked occasionally, however, e.g. to explain the not uncommon occurrence of nutritional anaemia in one of binovular twins reared under identical circumstances (Neale and Hawksley, 1933).

(3) By some the term constitutional is confined to those conditions in which there is definite evidence of something wrong with the make-up of the individual. This evidence consists in abnormalities of structure, familial hereditary or racial tendencies, or abnormalities of function that exist throughout life. Such conditions are familial acholuric jaundice, and the rarer form of anaemia called sickle-celled anaemia which is probably confined to the Negro race (Vaughan, 1936).

In the iron-deficiency anaemia of infancy, however, which has been investigated in the present work, although constitutional factors are often present, e.g. prematurity, rapid gain in weight, etc., the main aetiological factors are extrinsic, and it is in the correction of those extrinsic factors that we must attack the disease. Today we know the value of large doses of iron, and the relative ineffectiveness of small doses. Even so, and although iron metabolism has received a great amount of attention, there are as yet, many aspects of the subject awaiting explanation; nor is this surprising when the complexity

of the biochemistry of iron is realised. Studies in iron metabolism may be divided into three phases: absorption, storage, and utilization. At times it is convenient to consider the subject in the light of such a classification, but it must be remembered that often one phase is not independent of the others. It would seem, further, that in lower animals at anyrate, the action of copper is concerned with the metabolism of iron, since in the absence of copper, iron is found to be stored in the tissues but not utilized for the formation of haemoglobin (Cunningham, 1931). Copper has been claimed as a valuable adjunct to iron in the treatment of anaemia in infancy, but copper deficiency has not been demonstrated, and administered by itself copper has no action either on the formation of haemoglobin or on the reticulocyte percentage (Josephs, 1931; Parsons and Hawksley, 1933).

The present investigations have been directed towards the clearer definition of the means whereby the deficiency of iron arises; an attempt has also been made to assess the value of the measures used in the treatment of this type of anaemia, and to render more clear the processes by which cure is effected. To throw further light on these problems, patients have been investigated by means of balance studies so that all phases of the

treatment - retention, storage, utilization, and excretion - have been placed on an accurate quantitative basis. Thus, it has been rendered possible that observations be made on phenomena which have previously remained in the realms of conjecture. The results of these researches are presented under four headings; (1) the incidence of iron-deficiency anaemia in infancy, (2) the retention of inorganic iron in infancy and childhood, (3) the role of copper in iron-deficiency anaemia in infancy and childhood, (4) the influence of infection on the haematopoietic tissues.

## PART I. The Incidence of Iron-Deficiency Anaemia in Infancy.

The incidence of nutritional anaemia in infancy has aroused considerable interest in recent years, and its recognition and proper treatment is a matter of national concern. Since attention was drawn to the frequency of this form of anaemia during infancy by Mackay (1931) many workers have studied the problem, mainly from the aspects of diagnosis and treatment. Apart from the work of Mackay there have been few investigations into its incidence in this country (Davidson et al., 1935; Findlay, 1937; Fullerton, 1937; Colver, 1938). It is important that this aspect be further studied partly because of its importance in the social welfare of the infant and partly because of the light it throws on the aetiology of iron deficiency anaemia as a whole.

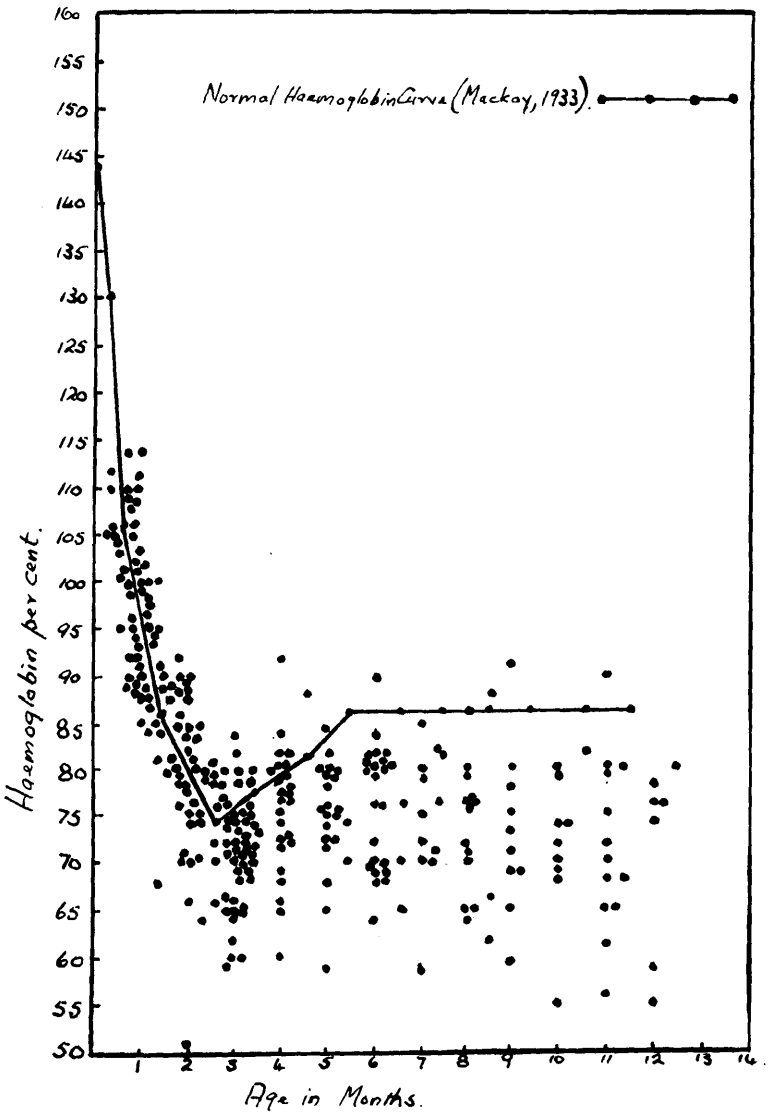
The present investigations were made to determine the incidence of the iron-deficiency anaemia of infancy in a large industrial area in Glasgow. Haemoglobin estimations were made on 300 infants of all ages up to one year. These infants were all under the supervision of a Welfare Clinic in Govan, a densely populated district. They did not receive iron supplements to their diets and were regarded by their mothers as healthy. The

haemoglobin estimations were all carried out by the same observer using a standardised Haldane haemoglobinometer. The blood was taken from the heel, the same two-edged stilette being used for every case.

The present results have been plotted against the curve suggested by Mackay (1931 and 1933)[2] as representing the normal haemoglobin levels for the first year of life (Graph I). The figures given by Mackay for normal values were obtained as follows: the values at ½ - 1 month, 1-2 months and 2-3 months were averages for groups of breast-fed babies living in their own homes; those for 3-4 months of age and upwards were based on haemoglobin values obtained with iron treatment, because it was assumed that these values would more truly represent the optimum than the lower values obtained at this age from babies without treatment. Joseph's (1936), criticises the view of Mackay (1931) that merely because iron raised the haemoglobin level, values below this should be regarded as indicativeof iron-deficiency. An effective reply would seem to be that not only does iron raise the haemoglobin level, it also diminishes the incidence of infection and increases the average gain in weight (Mackay, 1928); in short it assists in the maintainance of perfect health. Furthermore, the curve obtained by Merritt and

# GRAPH I.

HAEMOGLOBIN PERCENTAGES IN 300 INFANTS REFERRED TO AGE.

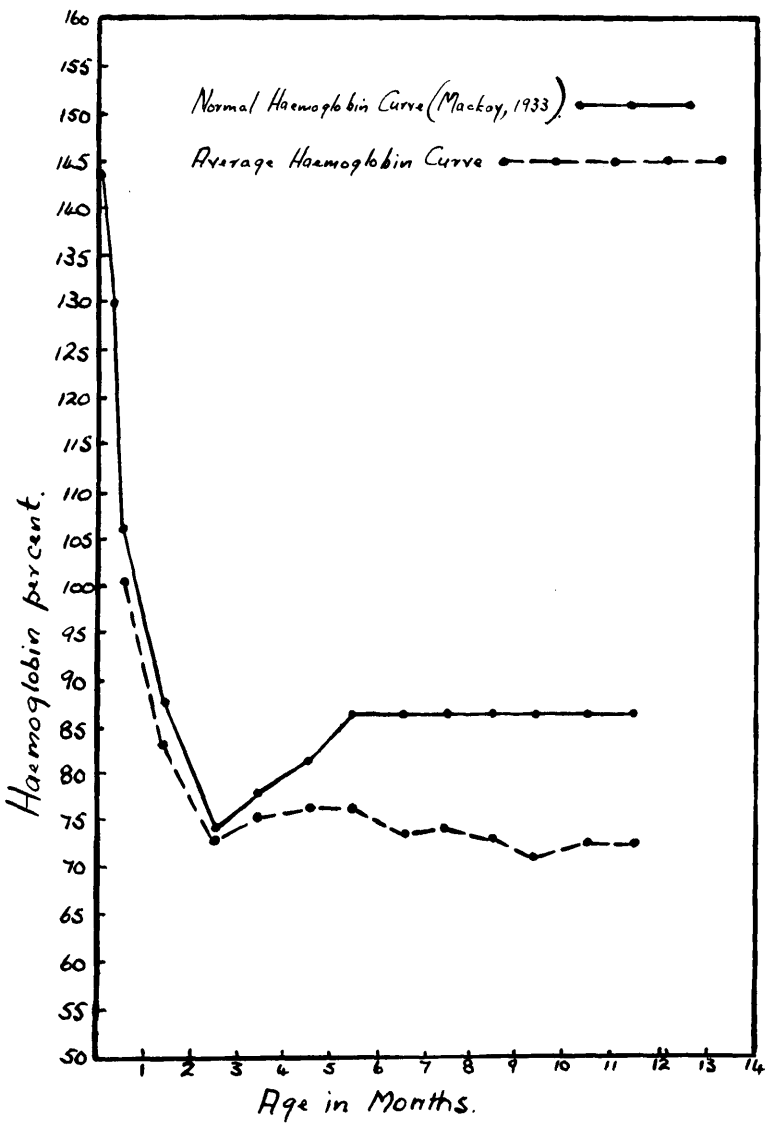


Davidson (1933) from haemoglobin values obtained from babies in New York City (where there is apparently little tendency for infants to become anaemic) is "strikingly parallel" to Mackay's standard normal haemoglobin curve.

In Graph I the results obtained in the present series of 300 infants are presented. The well-known trend of the haemoglobin level which falls rapidly during the first two months of life is clearly shown; it should also be noted that the great majority of haemoglobin estimations during the first $2\frac{1}{2}$ months of life fall into close proximity to the normal curve for that period. From the fifth month onwards, however, the individual values with few exceptions fall below the normal, frequently by as much as 15 to 30 per cent. This high incidence of anaemia among infants of the working class agrees closely with the findings of other workers in this country (Fullerton, 1937; Mackay, 1931; Colver, 1938). In Graph II is shown the average haemoglobin value at each month of age of the 300 infants studied. During the $\frac{1}{2}$ - 1 month age period the haemoglobin percentage averages 100.5 per cent; it falls rapidly maintaining a close relationship to the normal curve, so that by 2 - 3 months it reaches 72.5 per cent. For the next three months it rises slightly reaching an average level of 75.6 per cent at 5-6 months old. Thereafter it drops slowly to 71 per cent

# GRAPH II.

AVERAGE HAEMOGLOBIN VALUES FOR EACH MONTH OF THE FIRST YEAR OF LIFE.



at 9-10 months old and is constantly more than 10 per cent below the normal value of 86 per cent. These graphs show in a striking manner that it is only after the first six months of life that anaemia becomes so common. Taking a haemoglobin level of 10 per cent or more below Mackay's normal as indicating anaemia, only 25-3 per cent of infants under 3 months and 25.3 per cent in the 3-6 months ageperiod appeared to be anaemic, while in the age periods 6-9 months and 9-12 months, 70.7 per cent and 70.0 per cent respectively showed anaemia. These results are very similar to those of Mackay (1933)[2] and Fullerton (1937). They differ from those of Findlay (1937) who found no evidence of anaemia in sick children in the East End of London. Findlay's cases, however, were hospital patients including probably many who were underweight and in whom the haemoglobin values were likely to be higher than in rapidly growing infants of the same ages.

The present series contains no haemoglobin values for the first two weeks of life. It is known, however, that the haemoglobin level is high at birth - 143 per cent (Mackay, 1933)[2]; thereafter it falls rapidly till it reaches 106 per cent at ½-1 month. This fall is the result of haemolysis in response to the sudden rise in

oxygen tension of the infant's blood at birth (Mackay, 1933)[3], when the lung takes the place of the placenta as a medium of oxygenation.

As has been noted, the haemoglobin level continues to fall until about the third month of life, in some cases reaching remarkably low levels. (Graph I). There appears to be little doubt that this fall is due to continued haemolysis and must be considered physiological. That iron-deficiency does not play a part is shown by the following facts:- (1) Iron does not prevent the fall in haemoglobin during the first three months (Mackay, 1933)[3]; (2) the iron content of the liver increases up till the age of two months (Gladstone, 1932); (3) the reductions in red cells and haemoglobin are parallel and the blood does not show the low colour index of iron-deficiency (Merritt and Davidson, 1933); (4) if iron-deficiency were a factor, then babies under 3 months who grow most rapidly would show the lowest haemoglobin levels, which is in fact not the case (Mackay, 1931). Hampson (1928) found an excess bilirubinaemia until one month of age, and suggests that the haemolysis in the early weeks of life is controlled by the presence of an anti-haemolytic hormone. Josephs (1932)[1], however, regards the fall in haemoglobin to be due to diminution in the rate of haematopoiesis rather than to increased

destruction of the blood, putting forward as evidence the fact that administration of iron during the first three months of life has no influence on the reticulocytes, nor is it capable of arresting the fall in haemoglobin and red cells. Of possible significance in this connection is the finding of Trought (1932) that in the placenta and blood of the newborn infant there is a proportion of haemoglobin differing in some way from that of the adult. This "haemoglobin-alpha" persists in the circulation at least till 1½ months, i.e. during the time of the most rapid fall in haemoglobin. It is not known whether this "haemoglobin-alpha" has any effect on the resistance of the red cells to haemolysis. Of some interest is the finding (Table I) that by 2-3 months it is in babies of low birth weight that the haemoglobin has fallen to the lowest levels in spite of the fact that the lower the birth weight the higher the average haemoglobin level at birth (Mackay, 1933)(2). Thus babies of the lowest birth weight have the most rapid falls in haemoglobin values during the early months.

A likely explanation of the rise in haemoglobin values between the third and sixth months (Graph II) has been given by Gladstone (1932). He found that the iron content of the liver increased steadily till the age of two months while haemolysis was in progress. Thereafter it diminished

until at four months it had returned to the birth level. This is taken to indicate that after the age of 2-3 months when haemolysis is at an end, the stored iron is released for haemoglobin formation and that this store is exhausted about the age of six months, after which time iron-deficiency anaemia begins to appear. Mackay (1931 and 1933)[2] has shown that the administration of iron can produce a higher average value at four months than that obtained in the present series (Graph II), which suggests that sometimes the need for extra iron occurs as early as three months.

Following the slight rise in haemoglobin values during the 3rd, 4th and 5th months the curve falls gradually (Graph II). On the other hand the administration of iron from the age of two months produces an average haemoglobin value of 86 per cent (Mackay, 1933)[2] throughout the second six months of life, and the infants so treated show a lower morbidity rate. In view of these facts there can be little doubt that the low values obtained in the present series for infants over five months of age (Graph I) find their explanation in a deficiency of iron. There remains to discuss the explanation of this deficiency.

Deficient Ante-Natal Storage of Iron.

It is the view of many workers that though the foetus is able to draw upon the mother for all blood forming

materials for its own immediate needs, irrespective of the condition of the maternal blood, infants born to anaemic mothers are unable subsequently to maintain a normal haemoglobin level during the first year of life. (Strauss, 1933; Mackay, 1931; Paxton, 1936). The Birmingham workers are of the opinion that ante-natal storage of iron may be deficient to such a degree as to produce a congenital nutritional anaemia (Parsons, 1932; Parsons and Hawksley, 1933). This opinion appears to be based entirely on a history, from the mothers, of pallor at birth, and on the ability to produce an iron-deficiency anaemia in the newborn rat (Parsons and Hickmans, 1933). They put forward the suggestion that some mothers, who may not themselves exhibit hypochromic anaemia, have the power to retain their iron to the detriment of the foetus (Neale and Hawksley, 1933). Most authorities agree, however, that the haemoglobin level <u>at birth</u> bears no relation to maternal anaemia. Acting on this assumption and considering iron-deficiency anaemia from a quantitative aspect, Fullerton (1937) has concluded, from a convincing series of calculations, that the iron content of the newborn infant is independent of that of the mother. The same worker in a previous paper showed that the iron drain by the foetus is not sufficiently large to cause a marked

effect on the maternal iron content. (Fullerton, 1936).

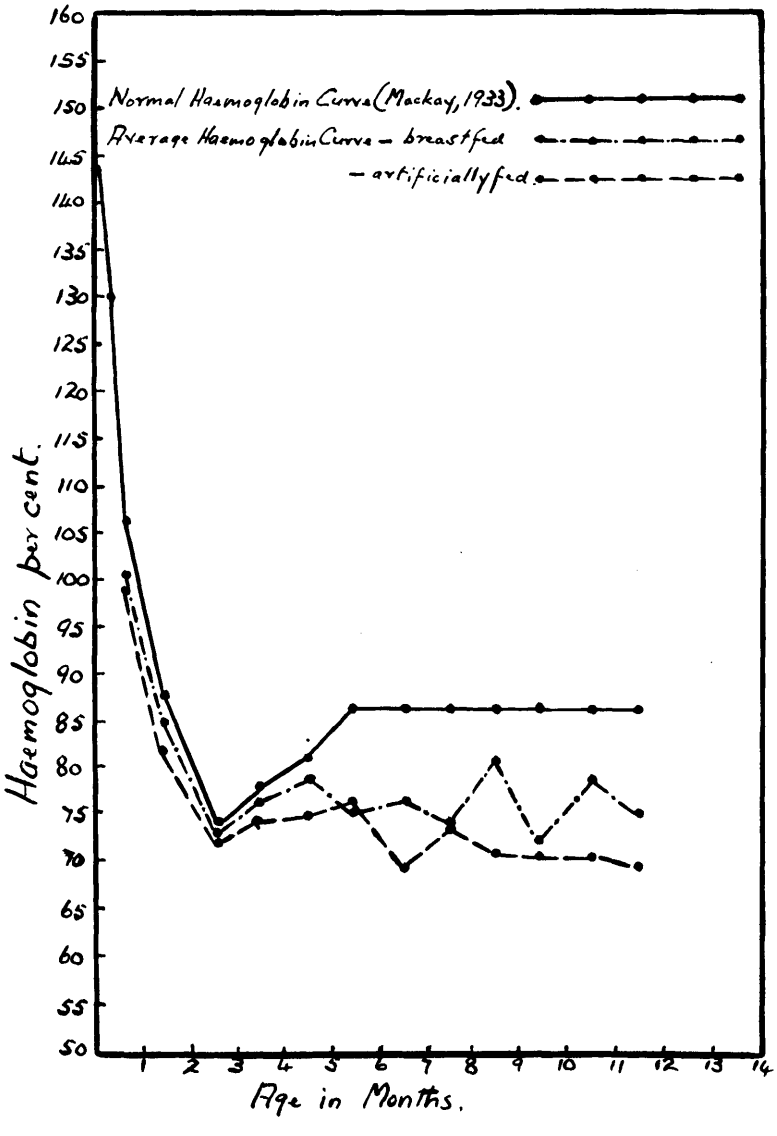
Deficient Post-Natal Supply.

It is generally agreed that the iron content of cow's milk is lower than that of breat milk (Peterson and Elvehjem, 1928; Cunningham, 1931; Wallgren, 1932) although there is marked variation in the figures obtained by different workers, and it seems to us that conclusions based on this supposition have as yet but poor foundations. Nevertheless in the present series the average haemoglobin curve obtained from breast-fed infants shows constantly higher values than the curve of the bottle-fed infants save for the 5-6 months age period (Graph III). The average value for the 8-9 months age period in the breast-fed group is seen to be 81 per cent; this figure is probably erroneous as only two of the breast-fed infants fell into this age group. This difference between the breast-fed and bottle-fed infants is less marked in the present series than was found by Mackay (1931) and Fullerton (1937).

While it has been suggested that the relatively high values found in the breast-fed infants are due to the higher iron content of human milk, it should be noted that this hypothesis does not explain why the bottle-fed

# GRAPH III.

## AVERAGE HAEMOGLOBIN VALUES OF BREAST-FED INFANTS COMPARED WITH VALUES FOR ARTIFICIALLY-FED INFANTS.



infants showed lower values as early as the age of ½-1 month, a time when the administration of iron does not affect the fall in the haemoglobin content of the blood. A possible explanation lies in the fact that there were more premature babies and infants of low birth weight (under 6 lbs.) in the first three months than during the rest of the year, and it is known that they have a more rapid fall in haemoglobin, are more likely to be bottle-fed, and have a lesser expectation of continued life. For this reason the curve for bottle-fed infants under three months was made up from a group containing more prematures and infants small at birth, than the group of breast-fed infants. Consequently the haemoglobin curve of the bottle-fed group tends to be lower than that of the breast-fed group. Josephs (1934) found a positive iron-balance of about 6 milligrammes per month in bottle-fed infants, and calculated that an exclusive milk diet cannot cause anaemia on the basis of its low iron content alone. Langostein and Edelstein found a similar retention of iron in infants fed on human milk. It would however seem that while the low iron content of milk, prolonged milk feeding and delayed institution of mixed feeding are likely to be potent factors in the production of iron-deficiency anaemia, there is little evidence to explain why the bottle-fed infant

should have a greater tendency to become anaemic than the breast-fed infant. At the same time the present results (Graph III) and those of others clearly demonstrate that this tendency is in fact present, and it is only possible to conclude that the anaemia is dependent in part on factors influencing iron utilization or iron storage which are not yet understood.

Birth Weight.

It was found in the present series (Table I) that during the second half of the first year of life, infants of low birth weight had on the whole a lower haemoglobin level than infants weighing 7 lbs. and upwards at birth.

TABLE I.

Average Haemoglobin Values for First Year of Life Referred to Birth Weight.

| Birth Weight. | ½ - 1 mths. | 1 - 2 mths. | 2 - 3 mths. | 3 - 4 mths. | 4 - 5 mths. | 5 - 6 mths. |
|---|---|---|---|---|---|---|
| Under 4 lbs. | --- | --- | 71.0 | --- | --- | --- |
| " 5 lbs. | 96.0 | 79.3 | 65.0 | 68.0 | --- | 68.0 |
| " 6 lbs. | 89.5 | 66.0 | 69.2 | 66.0 | 58.0 | 67.0 |
| " 7 lbs. | 102.3 | 81.7 | 72.5 | 76.0 | 73.3 | 75.8 |
| " 8 lbs. | 98.6 | 82.6 | 72.1 | 78.1 | 76.8 | 77.0 |
| Over 8 lbs. | 104.0 | 85.1 | 74.1 | 73.1 | 77.2 | 77.0 |

Table I (contd.)

| Birth Weight | 6 - 7 mths. | 7 - 8 mths. | 8 - 9 mths. | 9 - 10 mths. | 10 - 11 mths. | 11 - 12 mths. |
|---|---|---|---|---|---|---|
| Under 4 lbs. | --- | --- | --- | --- | --- | --- |
| " 5 lbs. | --- | --- | --- | 74.0 | --- | --- |
| " 6 lbs. | 58.0 | --- | 72.0 | --- | --- | --- |
| " 7 lbs. | 65.0 | --- | 58.0 | 68.0 | 77.5 | 72.0 |
| " 8 lbs. | 78.5 | 74.6 | 68.6 | 69.8 | 71.3 | 68.3 |
| Over 8 lbs. | 75.1 | 73.6 | 79.8 | 72.7 | 70.5 | 74.0 |

This finding is in agreement with those of other workers (Kunckel, 1915; Mackay, 1931; Abt and Nagel, 1932; Magnusson, 1935; Fullerton, 1937). The frequency of nutritional anaemia in premature infants and twins (Parsons and Hawksley, 1933) is a direct consequence of low birth weight. The tendency for the development of iron deficiency in infants small at birth depends on the fact that infants of low birth weight grow more rapidly in relation to birth weight than infants of normal weight, having at the same time a lower absolute iron content because of a smaller blood volume and a smaller iron store in the liver. The correlation between the haemoglobin level and the rate of growth has been stressed by Mackay (1931), Josephs (1934)

and Fullerton (1937). This also applies to premature infants and twins. It has been frequently stated, that as deposition of iron in the liver occurs most rapidly in the last three months of gestation (Hugounenq, 1899), early exhaustion of the foetal stores of iron explains the frequency of anaemia in premature infants in whom the period of iron storage has been cut short, and in twins where the available iron has to be shared. Gladstone (1932) has pointed out, however, that although the acquisition of iron by the foetus is greater during the last four months of intra-uterine life than during the first six, the foetal gain in weight during the last three months is greater than during the first seven. Thus during foetal life the increase in <u>total</u> iron content is not more rapid than the increase in total weight, so that premature babies are <u>in relation to their weight</u> almost as well supplied with iron as the full-time baby. It would appear that the greater tendency for anaemia to develop in babies small at birth is the result of relatively rapid growth in weight together with excessive haemolysis in the first 2-3 months of life (vide supra).

<u>Infections</u>.

The occurrence of a hypochromic anaemia in association with acute and chronic infections is well recognised

(Collins, 1935; Minat and Heath, 1932; Sturgis et al., 1935; Vaughan, 1936). Fullerton (1937) and Josephs (1934) are of the opinion that infection is an important aetiological factor in the iron-deficiency anaemia of infancy. In Table II are found the average haemoglobin values in the present series arranged according to whether or not there was a history of infections.

TABLE II.

Average Haemoglobin Values According to History of Infections.

| | 5 - 6 mths. | 6 - 7 mths. | 7 - 8 mths. | 8 - 9 mths. | 9 - 10 mths. | 10 - 11 mths. | 11 - 12 mths. |
|---|---|---|---|---|---|---|---|
| No history of infections. | 76 | 75 | 71.6 | 72 | 67.8 | 72.2 | 71 |
| History of infections. | 74 | 73.5 | 75 | 72.9 | 73.6 | 71.7 | 72.5 |

The infections comprised mainly mild gastro-intestinal and respiratory diseases and the exanthemata. Fifty-five per cent of the infants had a history of one or more infections. It is seen that there is little difference in the haemoglobin values of the two groups, a result which on first thought is distinctly surprising. The figures cannot be taken as reliable, however, as the histories of the infec-

tions were obtained from the mothers who were not all of even average intelligence. Attacking this problem from another aspect (vide infra) the author has found that severe infections have an adverse influence on the utilization of iron for haemoglobin synthesis, and that severe illnesses are frequently followed by anaemia, Mackay (1928) has observed an increased susceptibility to infections on the part of anaemic infants, and it seems probable that in this way a vicious circle may be established - infection causing anaemia and anaemia predisposing to infection. Fullerton's (1937) results leave little doubt that infections may cause marked falls in the haemoglobin level even during iron therapy.

Prophylaxis.

In view of the high incidence of iron-deficiency anaemia in working class infants the desirability of its prevention must be stressed, not so much on account of the anaemia *per se* but because of the associated morbidity and mortality. Mackay (1931) has found the administration of 4½ to 9 grains of Ferri et Ammon.Citras daily from the age of two months an effective measure in preventing anaemia and diminishing the incidence of infections. Alternatively, a dried milk to which iron has been added, e.g. Hemolac (Cow and Gate) may be used; this has the advantage of

ensuring that the infant always receives its iron supplement to the diet. Others have tried a more natural but effective prophylactic agent in the addition of foods of high iron content to the infant's diet at a more early age than has been customary in this country (Whitby and Britton, 1935). At the age of 4-5 months broth, vegetable purée, and the yolk of an egg may be added without harmful effect. It would be good if this method of early "mixed" feeding could be widely adopted.

Summary.

1. A study of 300 infants provides evidence of the prevalence of iron-deficiency anaemia during the first year of life. Twenty-six per cent of the total number of breast-fed infants and 35 per cent of the bottle-fed infants gave haemoglobin values of at least 10 per cent below Mackay's normal values for their respective ages. These precentages become much greater if infants under the age of six months be excluded.

2. The principle factors influencing the development of this type of anaemia are discussed. They are, (i) undue prolongation of exclusive milk diet, (ii) low birth weight, (iii) infections.

3. The importance of prophylaxis is stressed and methods of effecting this are discussed.

4. In the ensuing pages the curative treatment of iron-deficiency anaemia is discussed and some original observations are presented. Two factors frequently stated to have an adverse influence on the efficacy of iron therapy, viz. achlorhydria and infection have also been investigated from a new aspect.

## PART II. The Retention of Inorganic Iron in Infancy and Childhood.

The efficacy of iron therapy in nutritional or iron-deficiency anaemia is apparently dependent on the administration of large doses of iron if given orally (Heath, 1933; Witts, 1933). In this connection it is interesting to recall that the use of large doses was first advocated in 1832 by Blaud. Only a small amount of the iron ingested goes to the formation of haemoglobin and there has been much speculation as to the reason why it has to be given in such large doses, and as to the fate of the remainder of the iron which has not been utilized in haemoglobin building. That the body can store large amounts of iron has been shown by Reznikoff, Toscani, and Fullerton (1934), who found that, after the administration of phenylhydrazine hydrochloride to a case of polycythaemia, there was a blood destruction equivalent to 2.1 grammes of iron without any extra excretion of this metal. Ramage and Sheldon (1935) recovered as much as 50 grammes of iron from the tissues in a case of haemochromatosis. The necessity for the use of massive doses of inorganic iron salts has until recently caused many workers to doubt if iron deficiency is the sole factor in the aetiology of the nutritional

anaemias (Whipple and Robscheit-Robbins, 1930). Heath, Strauss, and Castle (1932) showed that the parenteral administration of iron in inorganic form resulted in 100 per cent utilization, and because of this Fullerton (1934) sees no reason to postulate the existence of another factor, and suggests that the necessity for large doses is dependent, among other things, upon imperfect absorption. There are, however, few observations regarding the absorption of iron, and the importance of further investigation on its fate after ingestion is obvious. With a view to throwing some light on this problem, a series of iron-balance studies has been conducted on six subjects, in-patients at the Royal Hospital for Sick Children in Glasgow.

Method of Investigation.

The subject was given a diet of known iron content for a period of four days before the beginning of the experiment. Thereafter, for a period of seven days on the weighed diet, the faeces were collected for analysis; then ferrous sulphate was added to the diet in doses which amounted to 4 grammes or 8 grammes of the sulphate, the equivalent of 803.45 milligrammes or 1606.90 milligrammes of iron per week, and the collection of the faeces continued. The ferrous sulphate was given in aqueous solution

with the addition of glucose syrup to minimize oxidation as recommended by Parsons and Hawksley (1933). The iron content of the urine was neglected, as all workers agree that urinary excretion of iron is negligible (Tompsett, 1934; Brock and Hunter, 1937).

In older children no difficulty was experienced in collecting the faeces so long as the children were confined to bed. Although of no great moment in iron metabolism studies, it was deemed desirable to collect the faeces uncontaminated by urine where possible, as this allowed of observations on the metabolism of other minerals, e.g. calcium and phosphorus, if necessary at a later date. To this end, for young male infants and children, a special metabolism bed as suggested by Findlay, Paton and Sharpe (1920-21) was used. Two short mattresses, about 10 inches thick, are placed at each end of the bed and separated from one another by a distance of eight inches. The child is firmly fixed so that the buttocks are suspended over the space between these mattresses. The child is supported and kept in position by means of a firm calico arrangement, to which the child is fixed, stretched taut between the top and bottom of the bed. The calico arrangement is composed of a body piece with a binder attached so that the trunk is controlled, and two leg pieces to which the legs and thighs

are bandaged with the legs held slightly apart. This piece of apparatus requires to be made to fit the child, special attention being paid to the size of the body piece and the width of the leg pieces. The fork is situated at the lower end of the sacrum, just at the upper end of the natal cleft. A blanket is lightly placed over the child with a wire cage on top, which keeps the weight of any extra bed-clothes off the child and the apparatus. The faeces are collected in a pouch of Jackinette lying in the space between the two mattresses. The penis is placed into the end of a small Paul's tube fixed to a piece of rubber tubing, and the urine thus drained directly into a separate vessel attached to the side of the bed. In this way the urine and faeces may be collected separately. It might be expected that a child confined in this way for periods sufficiently long to collect material for metabolism studies would be cross and irritable. Fortunately this is not the case, and the children so confined are usually quieter than the others in the ward.

Weekly determinations of the haemoglobin, red blood cells and colour index, were made in every case. The haemoglobin levels were determined by the Sahli method as this is more suitable for routine work than the Haldane method, and as it was not desired to compare the readings

with those of other workers as was the case in the observations in Part I of this thesis. The Sahli haemoglobinometer was standardised against blood, the iron content of which had been estimated, and it was found that a reading of 100 per cent. on the haemoglobinometer represented a haemoglobin content of 17.3 grammes per 100 cc. blood. After completion of the study, examination of the gastric contents, following a test-meal, was carried out in every case by the Rehfuss method, a Ryle's tube being used for aspiration of the gastric contents.

The total faeces for each period were placed in a porcelain evaporating dish (the weight of which was known), and treated with methylated spirits. Thereafter, the faeces were dried on a hot plate until constant weighings were obtained on three consecutive days, and the weight of the dried faeces thus obtained. The dried faeces were then finely divided by grinding in a mortar and stored in a stoppered glass jar. By calculating the iron content of aliquot samples of faeces from each period, the total iron output for each period was determined. The method used for determining the iron content of the faeces was that described by Taylor and Brock (1934), in which sulphuric acid digestion makes possible the volumetric estimation of iron by the permanganate titration method of

Margueritte (Treadwell and Hall, 1924) (see Appendix). Before the beginning of the metabolism studies the method was tested thoroughly and found to be accurate and satisfactory.

Like all macromethods for the determination of iron in biological material, this method is time-consuming, each estimation taking about eight days. When to this is added the ten to fourteen days required for drying the faeces it is about three weeks from the end of any one weekly experimental period before the determination of the iron retention during that period can be arrived at. This led to certain difficulties in planning experiments and could not be overcome by the adoption of any one of the many microcolorimetric methods for biological assay, because the errors entailed in those methods are greatly exaggerated when amounts of iron between 1 and 10 milligrammes are being estimated.

The results of iron-balance studies which have been carried out on six patients over long periods are presented. The duration of the experiments varied from eight to sixteen weeks and comprised in some cases as many as sixteen weekly periods. Of these patients, four gave normal or almost normal values for haemoglobin and red blood cells at the beginning of the metabolism studies, and the remain-

ing two were cases of severe hypochromic anaemia. The findings of the normal and anaemic cases are discussed separately.

Results: Non-anaemic Subjects.

Case 1. E.S., a girl aged ten years, ten months, was admitted to the wards with chorea minor. The iron-balance study, the results from which are shown in Table III, was begun after complete cessation of choreiform movements.

The first weekly period was in the nature of a control period during which no iron was administered apart from the small amount (37.87 milligrammes) contained in the food. Throughout the second, third, fourth, fifth and sixth periods, 4 grammes of ferrous sulphate (equivalent to 803.45 milligrammes of iron) were administered orally each week, the total iron intake of 841.32 milligrammes per period being the sum of the food iron and the inorganic iron given in the form of ferrous sulphate. The total iron output was not determined for the fourth and fifth periods. During the seventh, eighth, ninth and tenth periods the child received 8 grammes of ferrous sulphate (equivalent to 1606.90 milligrammes of iron) per week, the

total iron intake during each period being 1644.77 milligrammes. After completion of the tenth period the administration of ferrous sulphate was stopped.

From Table III it can be seen that this girl, who constantly presented a normal blood picture, retained remarkably large amounts of the iron administered: 72-87 per cent of the total intake per period was retained when the weekly intake amounted to 4 grammes of ferrous sulphate, and from 27-76 per cent of the intake with 8 grammes of ferrous sulphate. Another feature of interest is the wide variation of the retention of iron during different weekly periods. These differences are probably to be explained, in part, by the possibility that an apparently large retention during one period may be due to constipation with accumulation in the bowel of non-absorbed iron, which being passed in the faeces of the next period produces an apparently small retention of iron for that period. Thus, when there was a positive iron-balance of 1254.91 milligrammes during the ninth period, and a negative balance of 211.02 milligrammes during the tenth period, the amounts of faeces passed during these periods were 39.62 grammes and 112.20 grammes, respectively. This artefact could be abolished by the use of long periods

and it is of interest and significance in this connection that Brock and Hunter (1937) using three-day periods in contrast to the seven-day periods used in these experiments, found much larger variations in the retention of iron. From Table III it is also apparent that when the administration of ferrous sulphate was discontinued on completion of the tenth period, re-excretion of some of the iron retained took place during the eleventh, twelfth and thirteenth periods. In brief, the total amount of iron retained by the child over the fourteen weeks was approximately 3.5 grammes. The normal iron content of a child of this age is about 1.5 grammes, so that presumably the body stores of iron were trebled in amount.

Case 2. A.C., a boy aged nine years, ten months, was admitted suffering from subacute rheumatism. An iron-balance study (Table IV) was begun as soon as the sedimentation rate had fallen to normal. The first period was, as in the preceding case, a control period. From the second to the end of the seventh period 4 grammes of ferrous sulphate per week were administered, and from the eighth to the end of the twelfth period, 8 grammes per week. The blood picture was normal throughout the period of observation. During the

second period the retention of iron was equivalent to 51 per cent of the intake, while during the third and tenth periods, on the other hand, there were negative balances of 105.16 and 283.71 milligrammes respectively. It is obvious that the total retention of iron for the duration of the study was proportionately much less than was seen to occur in Case 1.

Case 3. G.N., aged seven months, was admitted with infantile atrophy. The results from the iron-balance study on this infant are shown in Table V. During the first six periods on metabolism the infant received orally 4 grammes of ferrous sulphate per week, the iron intake per weekly period being 820.11 milligrammes. The administration of ferrous iron was stopped during the last two periods of the experiment (that is the seventh and eighth), the iron intake of 16.66 milligrammes per period being derived in these periods from the food which consisted of cow's milk. The development of excoriation of the buttocks prevented the determination of the iron retention during the third, fourth and fifth periods. Table V shows that the infant retained large amounts of the iron ingested. The total iron retention in the body over the eight weeks of the metabolism study (at the rate shown in periods

one, two and six) amounted approximately to the remarkable figure of 2.5 grammes which is several times the amount of iron normally present in the whole body of an infant at this age. An accumulation of such magnitude raises the question as to the possibility of toxic effects resulting from saturation of the tissues with iron. No untoward symptoms were observed and it is of interest to note in passing that a careful perusal of the literature has brought to light only one authentic case of toxic effects following the administration of iron (Hurst, 1931).

Case 4. J. McE., aged eight months, was admitted with infantile atrophy. Table VI summarises the results obtained from an iron-balance study, lasting over a period of nine weeks. Throughout each of the first seven weekly periods 4 grammes of ferrous sulphate were administered orally. Thereafter the iron was omitted. This infant also retained large amounts of iron, although the retention did not approach the huge amounts recorded in Case 3 in spite of the fact, noted in Table VI, that the infant exhibited a mild hypochromic anaemia at the commencement of the study, whereas in Case 3 high haemoglobin values were obtained throughout.

Results: Patients with hypochromic anaemia.

Case 5. P.D., a boy aged three years, was admitted suffering from a profound hypochromic anaemia. The haemoglobin was 35 per cent., red blood cells 3,430,000 per c.mm., and colour index, 0.51. An iron-balance study was carried out while the boy was being treated with 4 grammes of ferrous sulphate per week. The results are tabulated in Table VII.

In this case, as in the others, the iron-content of the circulating haemoglobin as noted in the accompanying tables, was calculated by taking the blood volume to be $(\frac{\text{body-weight in kilogrammes}}{15})$ litres, and the iron content of the haemoglobin to be 0.335 per cent (Butterfield, 1909; Sachs, Levine and Appelsis, 1933). In this way the iron utilized for the formation of the new haemoglobin could be estimated, and the percentage of the retained iron so used calculated with a reasonable degree of accuracy (Table VII). It is observed that this patient retained a large percentage of the iron administered. It is also seen that during all the periods of observation only a small percentage of the large total iron retention was utilized in the building of new haemoglobin, the remainder being stored presumably in the liver as inert metal as far as haemoglobin

formation is concerned. This point is more forcibly brought home, if it is realized that between the first period and the end of the eighth period 2895.84 milligrammes of iron were retained, although the rise of 55 per cent. in the haemoglobin during that period represented the addition to an 0.8 litre circulation of 259 milligrammes of iron (equivalent to only 9 per cent of the total retention of 2895.84 milligrammes). These figures, obtained from observations over a much longer period, agree very closely with the figures published by Brock (1937) for similar experiments on adults suffering from idiopathic hypochromic anaemia.

Case 6. J.C., aged ten months, presented, on admission, to hospital, a picture of hypochromic anaemia of moderately severe degree. As in case 5, an iron-balance study was performed while the infant was under treatment for anaemia (Table VIII). In this case also the haemoglobin increase bore little relationship to the iron retained. The haemoglobin rose by 6 per cent in the seven days of the second weekly period (blood examinations being performed on the last day of each period); whereas the amount of iron retained by the body in those seven days (528.73milligrammes) would have allowed an increase to more than

100 per cent if the iron had been fully utilized for haemoglobin formation. The total amount of iron retained by this infant, during the second, third, fourth, fifth and sixth periods on metabolism, that is, during the time taken for the haemoglobin to rise from 50 to 85 per cent, was enough to produce a rise in the haemoglobin of more than 17 per cent per day during that same period of time. The actual daily rise in haemoglobin was only 1 per cent.

Relation of Gastric Acidity to Retention.

It has been shown by Heath (1933) that the haemoglobin and reticulocytes respond better to a given dose of iron when the gastric secretion is normal: he therefore draws the conclusion that the 'absence of free HCl in the gastric contents probably is a factor in preventing the adequate absorption of iron'. Mettier and Minot (1931) from a series of experiments with acid-buffered meat mixtures came to the following conclusions:- 'The studies appear to indicate that soluble iron compounds are absorbed from the gastro-intestinal tract or utilized more readily for blood formation when administered with acid than with alkaline meals.'

With these facts and ideas in mind it was decided to perform a test meal on every case at the completion of

CHART I.

RESULTS OF GASTRIC ANALYSIS IN CASE 1.

E.S. CHOREA.

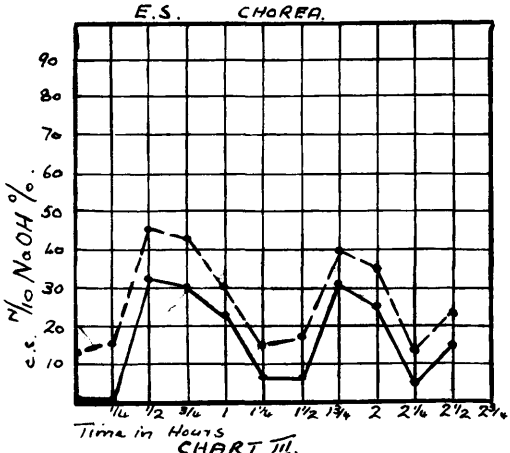



CHART II.

RESULTS OF GASTRIC ANALYSIS IN CASE 2.

A.C. RHEUMATIC INFECTION.

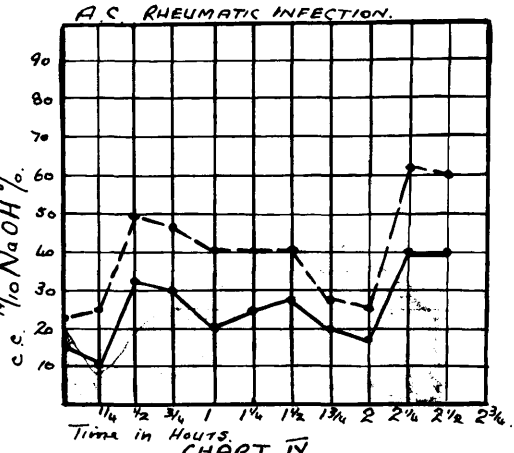



CHART III.

RESULTS OF GASTRIC ANALYSIS IN CASE 3.

G.N. INFANTILE ATROPHY.

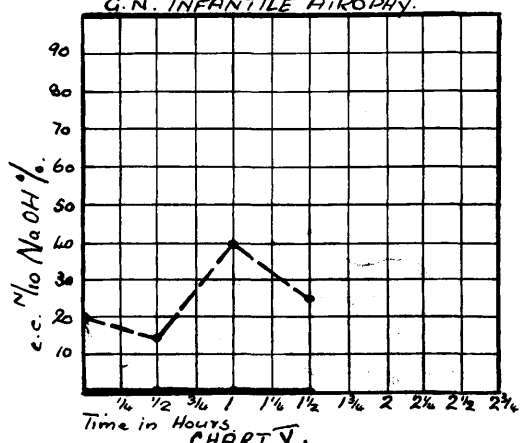



CHART IV.

RESULTS OF GASTRIC ANALYSIS IN CASE 4.

J. Mc E. INFANTILE ATROPHY.

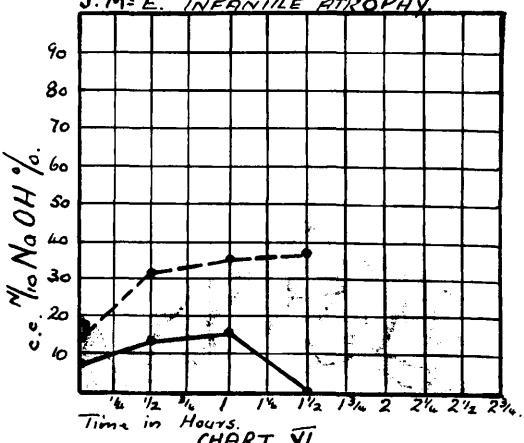



CHART V.

RESULTS OF GASTRIC ANALYSIS IN CASE 5.

P.D. NUTRITIONAL ANAEMIA.

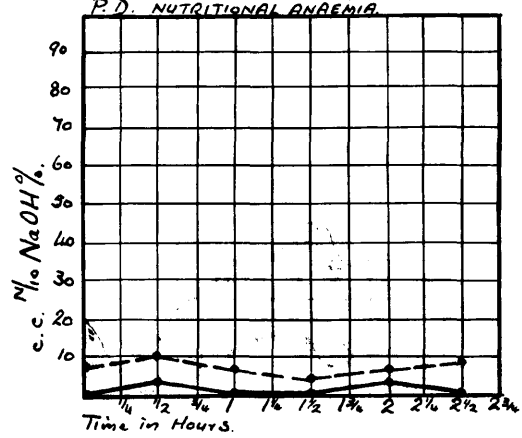
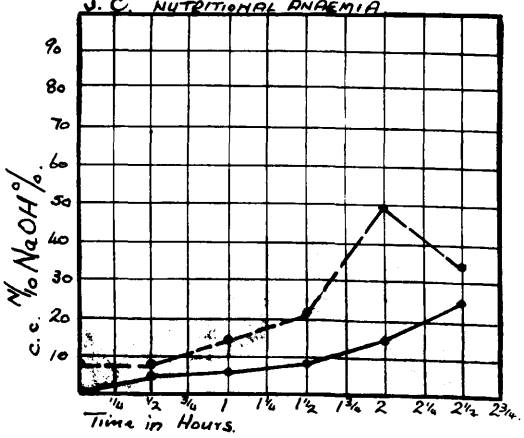



CHART VI.

RESULTS OF GASTRIC ANALYSIS IN CASE 6.

J.C. NUTRITIONAL ANAEMIA.




——— Free HCl. – – – – – Total Acidity.

the iron-balance study. The accompanying six charts depict the results of gastric analyses in the customary manner in the six cases already described. The numbers of the charts correspond to the cases to which they refer.

Scrutiny of the charts reveals certain interesting facts. Case 2, in which, it will be remembered, comparatively small amounts of iron were retained, showed a high gastric acidity. Case 3 which retained remarkably large amounts of iron over a period of eight weeks, showed not only the absence of free hydrochloric acid in the stomach under the conditions of the Rehfuss test meal, but its absence even after stimulation of the acid-secreting glands with histamine. This case, therefore, presented a true achylia gastrica in conjunction with the capacity to absorb large amounts of iron from the intestine. Cases 5 and 6 presented hypochlorhydria, a not uncommon finding in nutritional anaemia of infancy and childhood (Hawksley, Lightwood, and Bailey, 1934; Ogilvie, 1935). Case 5 absorbed enough iron in the first eight weeks of treatment to allow of all increase in haemoglobin of 13 per cent per day, and Case 6 it will be recalled absorbed enough iron to allow of an increase in haemoglobin of over 17 per cent per day.

It is realized that there is hardly justification

for drawing conclusions from so small a series of gastric analyses, but the results show that a low degree of gastric acidity does not preclude the ready retention of iron, which in the presence of anaemia may be used for haemoglobin building. Stewart (1937) has approached this question from a more clinical aspect, and is inclined to attach less importance than other workers to the diminished gastric acidity in the aetiology of the nutritional anaemia of infants (Faber, Mermod, Gleason, and Watkins, 1935). She concludes that it would appear extremely likely that the impairment of the gastric secretion is the result rather than the cause of the anaemia.

Discussion.

The results of the metabolism studies carried out in this series of cases demonstrate clearly that the body is capable of retaining remarkably large amounts of iron administered in the inorganic state. It is not possible, in view of these results, to assume that the necessity for large doses of iron in the treatment of nutritional anaemia is dependent on the poor absorption of iron-salts from the intestine. Other workers (Brock and Hunter, 1937; Reimann, Fritsch, and Schick, 1936) have formulated similar conclusions upon the results of iron-balance studies in adults.

It would appear also that high rates of iron retention

can be maintained over long periods, with resulting accumulation in the body of large amounts of iron. This was seen to be the case not only in the two patients showing anaemia, but also in three of the four non-anaemic patients. Even in Case 2 considerable amounts of iron were retained, although the total retention for the duration of the study was proportionately much less than was seen to occur in Case 1. The reason for this variation in the ability to retain iron is not obvious, but a similar observation has been made by Brock and Hunter (1937) and Reimann, Fritsch, and Schick (1936).

Apart from the fact that for a period of three weeks after the administration of ferrous sulphate was discontinued, Case 1 appeared to re-excrete some of the iron retained, there is no definite evidence forthcoming from the above results to indicate that the capacity to store iron is limited. Case 5 after ten weeks' administration of 4 grammes of ferrous sulphate per week, had retained 3086.05 milligrammes of iron, of which only 259 milligrammes were used in the synthesis of haemoglobin, and only 372.71 milligrammes of iron were excreted after discontinuance of the administration of iron on completion of the eleventh period, before a positive iron-balance was again established during the fifteenth period. Cases 3 and 4 after

retaining large amounts of iron over periods of six and seven weeks respectively, presented negative iron-balances for one weekly period only, after the stopping of massive iron-dosage; this can be explained by the fact that there is always a certain lag in the excretion of unabsorbed iron by the faeces.

It would appear, then, that the body is capable of storing in the tissues a large excess of iron over an indefinite period of time, and this again raises the question as to the possibility of toxic effects resulting. Polson (1933) maintained a high iron content in the livers of rabbits over periods of one to four years by means of repeated administration of iron subcutaneously and intraperitoneally and found no evidence of hepatic cirrhosis or pancreatic damage in any of the rabbits. He concluded that excess of iron in the body over long periods caused neither liver cirrhosis nor haemochromatosis in rabbits, and it is unlikely that excess of iron is responsible for the hepatic and pancreatic lesions of human haemochromatosis. Cappell (1930) after giving large doses of iron to mice and rats for as long as fourteen months found no evidence of haemochromatosis in the organs. The most outstanding contra-indication to the use of massive iron therapy can therefore be discounted.

It is necessary then to seek some other explanation of the need for such large doses, and of the inefficacy of smaller doses which only supply enough iron for haemoglobin formation. Brock (1937) suggests the possibility that large doses of iron are necessary in order to obtain a sufficiency of the copper impurity present in most iron preparations, having in mind the claims of some workers, that copper is effective as an adjuvant to iron in certain cases (Hart, Steenbock, and Elvehjem, 1928; Lewis, 1931; Josephs, 1931). Against this theory is the fact that the ferrous sulphate used in the metabolism studies here reported contained less than 0.0005 per cent of copper, so that no such interpretation can be put on the results of this series. Another possible explanation also advanced by Brock (1937) is that large quantities of iron may alter the chemical composition of the intestinal contents and thereby increase the absorption of some undetected haemopoietic factor. It is admitted, however, that there is no real evidence as to the existence of such a haemopoietic factor.

There is another explanation, however, which can be advanced as a result of the foregoing metabolism studies. Assuming that iron once deposited in the tissues is not available for the regeneration of haemoglobin, unless it

has been derived originally from the destruction of haemoglobin in the body, it follows that the great bulk of iron absorbed from the intestine during massive dosage with inorganic salts, would not be available as it would first find its way to the liver where it would be stored and thus rendered inactive for haemoglobin synthesis. It is reasonable to suppose then, that only by giving massive doses of iron can the rate at which it is stored by the liver be overcome and the excess iron be pushed past the liver directly into the blood serum, from whence it may be utilized for haemoglobin formation. This would explain the 100 per cent utilization of parenterally administered inorganic iron, as demonstrated by Heath, Strauss and Castle (1932), such iron presumably being absorbed directly into the systemic circulation, whereas orally administered iron is absorbed into the portal circulation and stored in the liver. Of interest in this connection also is the finding of Reicker (1930) that the level of the serum iron can be raised by the administration orally of large doses.

It is probable, however, that the final explanation of the reason why it is necessary to administer iron in amounts far in excess of the actual requirements for the regeneration of haemoglobin will only be attained when knowledge regarding the chemical combinations in which

iron occurs in the tissues is more precise, and also when a fuller understanding of the functions of the various components of the haemopoietic tissues is available.

Summary.

1. Observations on the retention of iron over long periods have been made in four non-anaemic and two anaemic children. The body is capable of retaining large amounts of iron. Only a small percentage of this iron is utilized for the synthesis of haemoglobin, even in those cases showing severe anaemia.

2. The necessity for giving large doses of iron by mouth does not depend on the poor absorption by the intestine.

3. No evidence was obtained that variations in gastric acidity influenced the retention of iron.

4. The suggestion is made that the iron stored in the liver is unavailable for haemoglobin formation, and only iron which overflows from the liver into the systemic circulation can be utilized for haemoglobin formation. Massive doses are necessary to effect this.

TABLE III.

ABSORPTION AND RETENTION OF IRON IN A NORMAL SUBJECT.

Case 1.E.S. Aet. 10.10/12 Years. CHOREA.

Wt. 24.72 Kilos. Blood Volume: $\frac{24.72}{15}$ = 1.65 Litres.

| Period | Intake mgm Fe | Output mgm Fe | Retention mgm Fe | % Retention | Hb(%) | R.B.C. | Iron (mgm) as Hb. | Iron (mgm) Retained as Hb | % of Retention Utilised | % of Intake Utilised. |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 37.87 | 4.08 | 33.79 | 90 | 90 | 5,100,000 | 871 | | | |
| 2 | 841.32 | 107.49 | 733.73 | 87 | 90 | 4,950,000 | 871 | Nil | Nil | Nil |
| 3 | 841.32 | 220.58 | 620.74 | 74 | 92 | 5,380,000 | 890 | 21 | 3.4% | 2.5% |
| 4 | 841.32 | - | - | - | 92 | 4,980,000 | 890 | Nil | - | - |
| 5 | 841.32 | - | - | - | 92 | 4,980,000 | 890 | Nil | - | - |
| 6 | 841.32 | 236.37 | 604.95 | 72 | 95 | 5,000,000 | 919 | 29 | 4.8% | 3.4% |
| 7 | 1644.77 | 699.03 | 945.74 | 57 | 95 | 4,970,000, | 919 | Nil | Nil | Nil |
| 8 | 1644.77 | 1177.26 | 467.51 | 27 | 95 | 5,190,000 | 919 | Nil | Nil | Nil |
| 9 | 1644.77 | 389.86 | 1254.91 | 76 | 95 | 4,960,000 | 919 | Nil | Nil | Nil |
| 10 | 1644.77 | 1855.79 | -211.02 | Nil | 95 | 5,270,000 | 919 | Nil | Nil | Nil |
| 11 | 37.87 | 1631.62 | -1593.84 | Nil | 95 | 5,040,000 | 919 | Nil | Nil | Nil |
| 12 | 37.87 | 783.77 | -745.90 | Nil | 95 | 5,070,000 | 919 | Nil | Nil | Nil |
| 13 | 37.87 | 107.28 | -69.41 | Nil | 95 | 5,020,000 | 919 | Nil | Nil | Nil |
| 14 | 37.87 | 23.58 | 14.29 | 37 | 95 | 5,130,000 | 919 | Nil | Nil | Nil |

## TABLE IV.

### ABSORPTION AND RETENTION OF IRON IN A NORMAL SUBJECT.

Case 2.A.C. Aet. 9.10/12 Years. RHEUMATISM.

Wt.20.79 Kilos. Blood Volume $\frac{20.79}{15}$ - 1.39 litres.

| Period | Intake mgm Fe | Output mgm Fe | Retention mgm Fe | % Retention | Hb(%) | R.B.C. | Iron mgm as Hb. | Iron (mgm) Retained as Hb. | % of Retention Utilised | % of Intake Utilised |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 39.48 | 105.13 | -65.64 | Nil | 85 | 4,880,000 | 683 | Nil | Nil | Nil |
| 2 | 842.93 | 410.21 | 432.72 | 51 | 85 | 4,570,000 | 683 | Nil | Nil | Nil |
| 3 | 842.93 | 948.09 | -105.16 | Nil | 85 | 4,780,000 | 683 | Nil | Nil | Nil |
| 4 | 842.93 | 633.92 | 209.01 | 25 | 90 | 4,960,000 | 727 | 44 | 21.1 | 5.2 |
| 5 | 842.93 | - | - | - | 90 | 5,120,000 | 727 | Nil | Nil | Nil |
| 6 | 842.93 | - | - | - | 90 | 5,000,000 | 727 | Nil | Nil | Nil |
| 7 | 842.93 | 585.38 | 257.55 | 30 | 90 | 4,750,000 | 727 | Nil | Nil | Nil |
| 8 | 1646.38 | 1055.97 | 590.41 | 36 | 90 | 5,010,000 | 727 | Nil | Nil | Nil |
| 9 | 1646.38 | 1583.45 | 62.93 | 4 | 90 | 4,870,000 | 727 | Nil | Nil | Nil |
| 10 | 1646.38 | 1930.09 | -283.71 | Nil | 90 | 5,230,000 | 727 | Nil | Nil | Nil |
| 11 | 1646.38 | 1577.74 | 68.64 | 4 | 90 | 5,160,000 | 727 | Nil | Nil | Nil |
| 12 | 1646.38 | 1644.80 | 1.58 | 0.9 | 90 | 5,020,000 | 727 | Nil | Nil | Nil |

## TABLE V.

ABSORPTION AND RETENTION OF IRON IN A NORMAL SUBJECT.

Case 3. G.N. Aet 7/12 Years. INFANTILE ATROPHY.

Wt. 4.68 Kilos. Blood Volume: $\frac{4.68}{15}$ 0.312 litres.

| Period | Intake mgm Fe | Output mgm Fe | Retention mgm Fe | % Retention | Hb(%) | R.B.C. | Iron (mgm) as Hb. | Iron (mgm) retained as Hb. | % of Retention Utilised | % of Intake Utilised |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 820.11 | 430.76 | 389.35 | 47 | 85 | 4,620,000 | 156 | | | |
| 2 | 820.11 | 207.12 | 612.99 | 75 | 85 | 4,660,000 | 156 | Nil | Nil | Nil |
| 6 | 820.11 | 317.65 | 502.46 | 66 | 85 | 4,950,000 | 156 | Nil | Nil | Nil |
| 7 | 16.66 | -81.59 | -64.93 | Nil | 85 | 4,730,000 | 156 | Nil | Nil | Nil |
| 8 | 16.66 | 12.91 | 3.75 | 22 | 85 | 4,740,000 | 156 | Nil | Nil | Nil |

## TABLE VI.

### ABSORPTION AND RETENTION OF IRON IN A NORMAL SUBJECT.

Case 4. J.McE. Aet. 8/12 Years. INFANTILE ATROPHY.

Wt. 5.94 Kilos. Blood Volume: $\frac{5.94}{15}$ 0.396 litres.

| Period | Intake mgm Fe | Output mgm Fe | Retention mgm Fe | % Retention | Hb(%) | R.B.C. | Iron (mgm) as Hb. | Iron (mgm) Retained as Hb. | %of Retention Utilised | % of Intake Utilised |
|---|---|---|---|---|---|---|---|---|---|---|
| *1 | 744.37 | 322.30 | 422.27 | 56 | 75 | 4,010,000 | 174 | | | |
| 2 | 821.09 | - | - | - | 70 | 4,490,000 | 163 | -11 | - | - |
| 3 | 821.09 | - | - | - | 80 | 5,450,000 | 186 | 23 | - | - |
| 4 | 821.09 | 612.05 | 209.04 | 25 | 85 | 5,150,000 | 197 | 11 | 5.3 | 1.3 |
| 5 | 821.09 | 641.00 | 180.09 | 22 | 90 | 5,100,000 | 208 | 11 | 6.1 | 1.3 |
| 6 | 821.09 | 730.79 | 90.30 | 11 | 90 | 5,100,000 | 208 | Nil | Nil | Nil |
| 7 | 821.09 | 626.04 | 195.05 | 24 | 90 | 4,750,000 | 208 | Nil | Nil | Nil |
| 8 | 17.64 | 104.72 | -87.08 | Nil | 90 | 4,970,000 | 208 | Nil | Nil | Nil |
| 9 | 17.64 | 9.27 | 8.37 | 45 | 90 | | | | | |

* The total iron intake per period is less than those given for the other periods up to the seventh. This is due to the fact that the infant vomited two doses of iron during the period, and thus the figures given for this period are less accurate than is the case in the other periods.

## TABLE VII.

ABSORPTION AND RETENTION OF IRON IN A CASE OF NUTRITIONAL ANAEMIA.
Case 5. P.D. Aet. 3 Years. NUTRITIONAL ANAEMIA.
Wt. 12.00 Kilos. Blood Volume - 12/15 Litres - 0.80 Litres.

| Period | Intake mgm Fe | Output mgm Fe | Retention mgm Fe | % Retention | Hb(%) | R.B.C. | Iron (mgm) as Hb. | Iron (mgm) Retained as Hb. | % of Retention Utilised | % of Intake Utilise |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 23.31 | 20.23 | 3.08 | 13 | 35 | 3,430,000 | 163 | | | |
| 2 | 826.76 | 346.80 | 479.96 | 58 | 39 | 3,670,000 | 196 | 33 | 6.9 | 4.0 |
| 3 | 826.76 | 385.89 | 440.87 | 53 | 52 | 4,360,000 | 244 | 48 | 10.9 | 5.8 |
| 4 | 826.76 | 339.32 | 487.44 | 59 | 63 | 5,040,000 | 295 | 51 | 10.5 | 6.2 |
| 5 | 826.76 | 245.76 | 581.00 | 70 | 78 | 4,980,000 | 365 | 70 | 12.0 | 8.4 |
| 6 | 826.76 | 178.29 | 638.47 | 77 | 85 | 5,290,000 | 399 | 34 | 5.3 | 4.1 |
| 7 | 826.76 | 584.64 | 242.12 | 27 | 87 | 5,000,000 | 405 | 6 | 2.5 | 0.7 |
| 8 | 835.51 | 812.61 | 22.90 | 3 | 90 | 5,070,000 | 422 | 17 | 73.9 | 2.0 |
| 9 | 835.51 | 676.18 | 159.33 | 19 | 90 | 5,070,000 | 422 | Nil | Nil | Nil |
| 10 | 835.51 | 1113.15 | -277.64 | Nil | 90 | 5,010,000 | 422 | Nil | Nil | Nil |
| 11 | 835.51 | 523.91 | 311.60 | 37 | 90 | 5,050,000 | 422 | Nil | Nil | Nil |
| 12 | 32.06 | 390.41 | -358.35 | Nil | 90 | 5,070,000 | 422 | Nil | Nil | Nil |
| 13 | 32.06 | 33.69 | -1.63 | Nil | 90 | 5,010,000 | 422 | Nil | Nil | Nil |
| 14 | 32.06 | 44.79 | -12.73 | Nil | 90 | 5,000,000 | 422 | Nil | Nil | Nil |
| 15 | 32.06 | 24.47 | 7.59 | 24 | 90 | 5,020,000 | 422 | Nil | Nil | Nil |
| 16 | 32.06 | 17.86 | 14.20 | 44 | 90 | 5,040,000 | 422 | Nil | Nil | Nil |

## TABLE VIII.

### ABSORPTION AND RETENTION OF IRON IN A CASE OF NUTRITIONAL ANAEMIA.

Case 6. J.C. Aet. 10/12 Years. NUTRITIONAL ANAEMIA.

Wt. 9.31 Kilos. Blood Volume: $\frac{9.31}{15}$ - 0.62 litres.

| Period | Intake mgm Fe | Putput mgm Fe | Retention mgm Fe | % Retention | HB(%) | R.B.C. | Iron (mgm) as Hb | Iron (mgm) Retained as Hb. | % of Retention Utilised | % of Intake Utilised |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 18.18 | 5.62 | 12.56 | 61 | 50 | 4,580,000 | 182 | | | |
| 2 | 824.66 | 295.93 | 528.73 | 64 | 56 | 4,320,000 | 204 | 22 | 4.1 | 2.6 |
| 3 | 824.66 | 224.54 | 600.12 | 73 | 65 | 5,050,000 | 236 | 32 | 5.3 | 3.9 |
| 4 | 824.66 | 522.35 | 302.31 | 37 | 73 | 5,000,000 | 265 | 29 | 9.6 | 3.5 |
| 5 | 824.66 | 429.86 | 394.80 | 47 | 83 | 5,080,000 | 301 | 36 | 9.1 | 4.4 |
| 6 | 824.66 | 411.88 | 412.78 | 49 | 85 | 5,040,000 | 309 | 8 | 1.9 | 0.9 |
| 7 | 824.66 | 354.95 | 469.71 | 57 | 85 | 5,080,000 | 309 | Nil | Nil | Nil |
| 8 | 824.66 | 537.66 | 287.00 | 35 | 85 | 5,030,000 | 309 | Nil | Nil | Nil |

## PART III. The Role of Copper in Iron-Deficiency Anaemia in Infancy and Childhood.

Recent investigations have yielded conflicting results as regards the efficacy and mode of action of copper in the treatment of the hypochromic group of the anaemias. Research in this subject has been along two lines, comprising on the one hand animal experiment, and on the other the trial use of copper in the treatment of anaemia occurring in the human subject. In 1925 Hart, Steenbock, and Elvehjem, and Waddell showed that iron alone was inadequate to bring about regeneration of haemoglobin in rabbits, previously rendered anaemic by milk feeding. On the other hand, fresh cabbage or iron-free chlorophyll in the presence of inorganic iron ($Fe_2O_3$) rapidly cured the anaemia. In 1927 Hart, Elvehjem, Waddell, and Herrin demonstrated that Vitamin E was not the factor supplied by the cabbage, etc., and that while pure ferrous sulphate was ineffective, impure ferrous sulphate was quite efficient, a fact which may explain a large number of the discrepancies in the results of other workers. Having demonstrated (Waddell, Steenbock, Elvehjem, and Hart, 1928)[1] the suitability of the rat for studies in anaemia, they further showed that the other factors necessary in addition to iron were of an inorganic nature, because the ashed residues from dried

beef liver, dried lettuce, and yellow corn were effective in curing anaemia (Waddell, Elvehjem, Steenbock, and Hart, 1928(2)). Their subsequent work on the rat, scrupulously controlled at every step, has shown that for the cure of "milk anaemia" both iron and traces of copper are essential (Hart, Steenbock, and Elvehjem, 1928; Waddell, Steenbock, and Hart, 1929(1), 1929(2); Waddell, Steenbock, Elvehjem, and Hart, 1929). The same holds good for an analogous anaemia in chicks given milk and a cereal low in iron and copper (Elvehjem and Hart, 1929).

That copper occurs in vegetable materials used as foodstuffs had, of course, been known for some time (Meissner, 1816; Maquenne and Demoussy, 1920; Guérithault, 1920) but until 1928 no physiological function had been assigned to it.

McHargue, Healy, and Hill (1928), Becker and McCollum (1930), Keil and Nelson (1931), Wickwire, Burge, and Krouse (1936), Stein, Radetsky, and Lewis (1936) and Lewis, Weichselbaum, and McGhee (1930) have all confirmed this effect of copper. Beard, Myers, and Shipley (1929) and Mitchell and Miller (1931) have claimed, however, that purified inorganic iron alone would slowly cure the anaemia of the rat, and Hart, Elvehjem, Steenbock, Kemmerer, Bohstedt, and Fargo (1929) have demonstrated that in the

nutritional anaemia of suckling pigs, iron alone stimulates haemoglobin synthesis as well as iron supplemented with copper. They suggest that this may be due, either to the fact that their pigs had some undetected available source of copper, or that in pigs an iron deficiency occurs before there is any copper deficiency. Rohscheit-Robbins and Whipple (1930) have found that the action of copper alone in curing anaemia in dogs is uncertain, and without question is far less potent than iron. Their conclusions are, however, not directly comparable with those of the Wisconsin workers, because the former produced the anaemia by repeatedly bleeding the dogs, whereas the "milk anaemia" of the rats used by the latter workers was a true nutritional anaemia produced by a deficiency of food factors. Further support is given to those who stress the importance of copper in haemoglobin synthesis, by the finding that the anaemia which is common among yearlings and heifers' suckling calves in Florida responds rapidly to ferrous ammonium citrate fortified with copper sulphate, but that iron supplements alone have proved inadequate (Neal, Becker, and Shealy, 1931).

In 1928, Tikes, Cave, and Hughes produced evidence which seemed to indicate that, in the rat, manganese added to a milk-iron diet gave almost as good results as did

copper given in the same way, and they suggested that there was a group of substances, rather than a single substance, which is active in haemoglobin building. These workers later confirmed their original contention (Titus and Hughes, 1929) and showed that the same applied to the rabbit (Titus, and Cave 1928). Beard, Myers, and Shipley (1929) also found that cobalt, nickel and germanium were as good supplements as copper, and later added to these several other elements possessing similar properties, e.g. arsenic, manganese, titanium, zinc, selenium, etc. (Beard and Myers, 1930). Nevertheless, Lewis, Weichselbaum, and McGhee (1930) obtained no haemoglobin regeneration where iron plus manganese or iron plus cobalt were fed to rats, although they confirmed the potency of copper in this respect, and Krauss (1931) obtained no better results with iron plus copper plus manganese than with iron plus copper. Keil and Nelson (1931) found the following elements to be inactive as supplements to iron in the treatment of "milk anaemia" in the rat; vanadium, titanium, manganese, nickel, arsenic, germanium, zinc, chromium, cobalt, tin, and mercury, although they too confirmed the potency of copper in this respect. Waddell, Steenbock, and Hart (1929)[2] reported no success with twelve "trace" elements - zinc, chromium, germanium, nickel, cobalt, mercury, lead, antimony, tin,

cadmium, arsenic, and manganese - and concluded that copper is unique in this connection and must be considered a necessary element in the nutrition of the animal body. Robscheit-Robbins and Whipple (1930) tested the effect on anaemic dogs of various mixtures of copper, zinc, aluminium iodine and phosphates, with and without iron, and rarely observed any increase of haemoglobin production above that to be expected from iron alone.

It is clear that animal experiments regarding the efficacy of copper as an adjuvant to iron in the treatment of anaemia have yielded conflicting results. The weight of evidence suggests, however, that copper does play some part in haemoglobin synthesis, and justifies its trial as a therapeutic agent in the anaemias of the human subject. There would not appear to be sufficiently convincing evidence to justify the inclusion in the haematologist's pharmacopoeia of the other "trace" elements mentioned.

There are several interesting analogies between iron and copper. McHargue (1925) demonstrated that the new born calf has a store of copper in its dried liver at birth about eight times as large, weight for weight, as that which he found in the adult animal's liver, and also that on the same basis, the amount of copper in the body of a new born rat is nearly double that in the adult rat. It

appears, moreover, that young guinea-pigs have no copper or iron stores (McHargue, 1925). That a considerable storage of copper occurs in intra-uterine life has been shown in the case of the human being as well as in lower animals (Morrison and Nash, 1930; Cunningham, 1931), and analyses of infant cadavers have revealed that smaller amounts of copper are found in the livers of anaemic infants than in those of non-anaemic infants (Chou and Adolph, 1935). Furthermore, Lindow, Elvehjem,and Peterson (1929) have shown that cow's milk is low in copper as well as in iron.

These points of resemblance in the storage of iron and copper in the human infant, suggest that nutritional anaemia may, in some cases at least, be due in part to a copper deficiency, having in mind the animal experiments quoted above. As Hawksley (1934) has pointed out, two methods have been employed to demonstrate the action of copper in nutritional anaemia. One consists in prescribing iron and copper together and comparing this with iron alone; the other consists in using iron alone, and in cases not cured then adding copper. Parsons and Hawksley (1933) in a large series of nutritional anaemias, found three cases in which there was no haemoglobin response after at least six weeks of iron therapy. In each, using copper as a

supplement, cure was obtained. These facts raise the question as to whether copper, being a frequent impurity in iron salts, has not played a part in the good results previously reported from iron medication. Sheldon and Ramage (1932) have shown that the distribution of copper in preparations of iron appears to be entirely inconstant. On the other hand, Davidson (1933), although accepting the conclusions of Waddell, Elvehjem, Steenbock, and Hart (2) (1928) as to the necessity of copper as a supplement to iron in the rat, does not regard copper as of value in the human subject. In 100 Aberdeen families of the poorest classes he found that the diet contained at least 4.6 milligrammes of copper per diem, and in these circumstances it was difficult to believe that small amounts of copper added to iron could be of much value. To infants, however, fed on a diet of cow's milk, which contains only very small amounts of copper, Davidson's results are not necessarily applicable.

Josephs (1931) gave iron and copper in one series of anaemic infants (aged 3 months to 2 years) and iron only in another, and found a more rapid recovery in the former group. He concluded that for a maximum rise in haemoglobin, some factor is needed in addition to iron, and that in most cases this factor is not sufficiently supplied

by the food, but is supplied by copper. A complicating factor in Josephs' series was the large number of infants with infections such as pneumonia, empyema, tuberculosis, syphilis, etc. Lewis (1931) found that iron and copper given in combination to children with nutritional anaemia was more effective than iron alone. Similar results have more recently been reported by other workers (Goldstein, 1935; Elvehjem, Duckles, and Mendenhall, 1937). Mills (1930) obtained similar results in adults, which are difficult to reconcile with Davidson's (1933) estimation of the copper content of the adult diet. Against these findings is the work of Mackay (1933)[1] who, noting the fact that "milk anaemia" in infants is similar to "milk anaemia" in rats, was unable to obtain evidence that in the former copper is a deficiency as it would appear to be in rats. She concluded (Mackay, 1933)[1] that although copper deficiency may occur in isolated cases of nutritional anaemia it plays no part in the great majority, though she admitted the possibility that the finding of many workers that iron alone is ineffectual may be due to the use of iron of absolute chemical purity, i.e. free from other heavy metals such as copper (Mackay, 1931). The iron preparation used in Mackay's (1933)[1] own series was not copper-free. Lottrup (1934) found copper to be ineffec-

tive in the treatment of anaemia in children, but only quoted two cases.

Usher, MacDermott, and Lozinski (1935) tested the efficacy of copper as a prophylactic agent against simple anaemia of infancy, making observations on 233 infants in an institution for foundlings. To one group they gave only iron, to a second copper and iron, and the third group was a control. They found that at the age of one year, the copper and iron group had a haemoglobin of 19 per cent above that of the control group, while the group receiving iron alone had a haemoglobin of only 15 per cent above the control. The copper also produced advantageous results by increasing the resistance to infections and reducing the mortality rate, which was over 14.5 per cent for the control children, 11.6 per cent for those receiving iron only, and 6.3 per cent for those given the additional copper. Muller (1935) stated that in mild and moderately severe anaemia in children he could obtain cure in three to four weeks with copper alone, but this finding is contrary to the results of all British and American workers. Görter (1931) brings forward some evidence, regarding the action of copper, from a different angle, finding that the copper content of the blood in infants suffering from nutritional anaemia was twice as high as at birth, or as

that found in control convalescent children. This he suggests may mean that the copper in the body has been mobilised in response to the need for haemoglobin; alternatively he admits that this finding may be interpreted as indicating that in those anaemias there is no shortage of copper. The same worker (Görter, 1933) has found a high percentage of copper in the blood in pregnancy and infections diseases, suggesting that as both conditions necessitate increased production of haemaglobin there is in consequence a mobilization of copper.

These clinical researches into the function of copper as a haemopoietic factor have, as was also seen to be the case in animals, yielded conflicting results. It would appear probable, however, that copper in some form is necessary for normal erythropoiesis. Further research has been carried out with a view to determining at what stage copper acts in the process of maturation of the erythrocyte, and as to its mode of action.

Mode of Action of Copper.

Elvehjem, Steenbock, and Hart (1929) have demonstrated that the haemoglobin of rat blood does not contain copper as part of its molecule. It has been further shown (Elvehjem, 1932; Elvehjem and Sherman, 1932) that although in rats the cure of anaemia was not produced by inorganic iron,

these salts were readily assimilated and stored in the liver and spleen; if copper was subsequently given the iron in the liver was removed and built into haemoglobin. From these results Elvehjem concluded that copper does not function in the assimilation of iron, but plays a part in the conversion of the iron into forms which can be used for the construction of the haemoglobin molecule. Muntwyler and Hanzal (1933) also concluded that copper, when given to anaemic rats, can mobilize the iron stored in the liver to produce haemoglobin and increase the red blood corpuscles. Parsons (1933) has pointed out that in nutritional anaemia of infancy, copper may in resistant cases build haemoglobin, but has no effect on the reticulocytes or the red cell count, indicating that although copper can affect haemoglobin synthesis it has no effect on the "stroma substances". A similar conclusion was reached by Josephs (1932).(2) These facts lead to the conclusion that copper speeds up a synthetic reaction already in progress, which is probably of a catalytic nature (Vaughan, 1936). Recognizing the fact that copper is active in combining with nitrogenous compounds, and that in haemocyanim, the blood pigment occurring in several invertebrates, copper takes the place of the haemoglobin molecule, the idea of copper acting as a catalyst seems a rational one (McGowan, 1930). Further, Cunning-

ham (1931) has shown in the case of rats that copper definitely increases the proportion of "organic" to total iron in the liver, and he considers that this change from "inorganic" to "organic" iron is probably a step in haemoglobin building dependent on the presence of copper. He also suggests that any modification of the state of iron in this direction would probably be a change from "inorganic" iron to an iron porphyrin and that it is possible that copper may promote this change. He makes an entirely speculative, but interesting, suggestion as to the process whereby copper may bring about the formation of an iron porphyrin. Turacin, the blood pigment of certain South African birds, is a naturally occurring copper porphyrin, and by chemical manipulation the copper can be displaced by iron, giving a compound practically indistinguishable from haematin. He suggests that the effect of copper in increasing the proportion of "organic" iron is achieved by a preliminary formation of a copper porphyrin, and subsequent replacement of copper by iron. This would fit in with the observations outlined above and with the general chemical nature of the elements.

Present Investigation.

The present series of researches was conducted in an effort to ascertain whether copper had any function in erythropoiesis in the human subject, and, if so, its mode of

action. It has already been shown (Part II) that pure inorganic iron alone is capable of curing the nutritional anaemia of infancy and childhood. Nevertheless, it was thought to be a reasonable assumption that if copper did play some part in haemoglobin synthesis, treatment by iron and copper would be more effective than by iron alone. In the iron deficiency anaemia of infancy at any rate, it would appear probable that the factors concerned in the production of iron deficiency, prominent amongst which are (1) low birth weight, (2) artificial feeding, and (3) delayed institution of mixed feeding (Fullerton, 1937; Neale and Hawksley, 1933; Mackay, 1931) are likely also to bring about the existence of a copper deficiency, bearing in mind the analogies existing in the distribution of iron and copper in tissues and food.

Method of Investigation.

Nine cases of nutritional anaemia in infancy and childhood have been studied. Iron and copper were used in the treatment of six of the cases in the following way. After four days on a diet of known iron content, inorganic iron as ferrous sulphate was given orally for periods of one to three weeks. This was then discontinued and the patients observed for varying periods until the haemoglobin values of the blood became constant, or showed no appreciable

tendency to rise. When copper, as cupric sulphate, was given orally and the effect on the haemoglobin recorded. The ferrous sulphate was given in doses of 4 or 8 grammes per week (equivalent to 803.45 or 1606.90 milligrammes of iron). It was dissolved in water with the addition of glucose syrup to prevent oxidation (Parsons and Hawksley, 1933) and contained less than 0.0005 per cent of copper. Copper sulphate ($CuSO_4$) was given in doses of 40 milligrammes per week, save in one case where the dose was 30 milligrammes per week. In the remaining three cases ferrous sulphate was given in doses of 0.5 grammes per week for several weeks, in an attempt to produce an iron storage in the body without changing the haemoglobin level. This attempt was completely successful only in one case. In all three cases when the haemoglobin levels had remained constant for several weeks the iron medication was stopped for a variable time and then copper sulphate, in doses of 40 milligrammes per week, was given and any mobilization of the stored iron for haemoglobin formation calculated.

Weekly determinations of the haemoglobin and red cells were made in every case. The haemoglobin levels were determined by the Sahli method using a standardised haemoglobinometer (Part II). The iron metabolism of each patient was demonstrated by a balance study (divided into seven-day

periods) over the time during which iron was administered, and until a positive iron balance was re-established following discontinuance of the iron administration. In this way the amount of iron retained in the body was estimated, and the percentage of retained iron utilized for the formation of new haemoglobin calculated with a reasonable degree of accuracy. The iron content of the circulating haemoglobin was calculated by taking the blood-volume to be $\left(\frac{\text{body-weight in kilogrammes}}{15}\right)$ litres, and the iron content of the haemoglobin to be 0.335 per cent (Butterfield, 1909; Sachs, Levine, and Appelsis, 1933). The technique used in the conduction of these balance studies has been described in Part II of this thesis.

Case Reports.

Case 7. M.G., aged one year ten months. Nutritional Anaemia and upper respiratory infection.

This girl was admitted with severe nutritional anaemia (haemoglobin 31 per cent); and an acute upper respiratory infection necessitated the transfusion of 80 ccs. citrated blood.

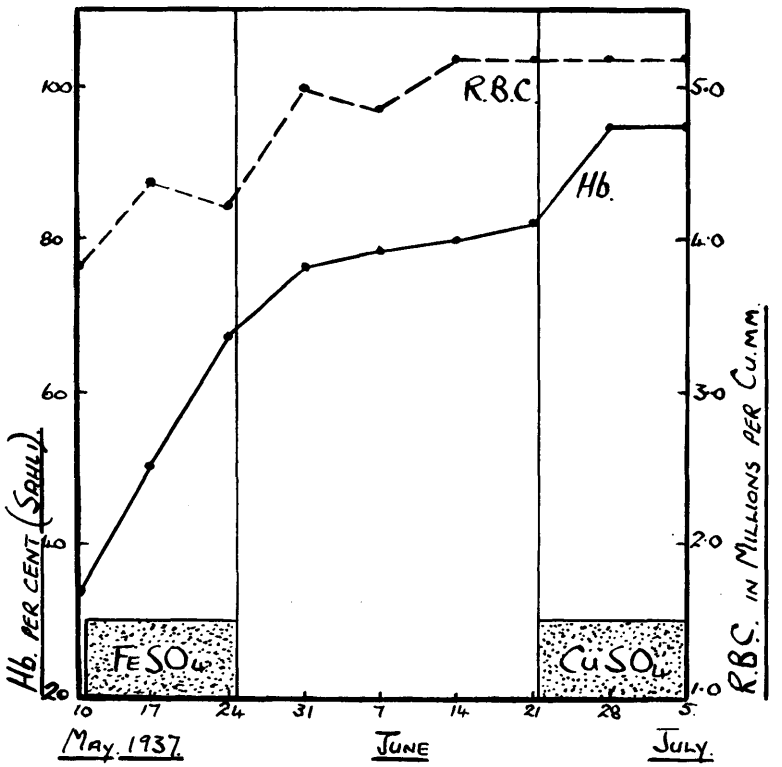
| | Haemoglobin per cent. | Red blood cells c.mm. | Colour Index. |
|---|---|---|---|
| Before transfusion, | 31 | - | - |
| After transfusion, | 37 | 3,760,000 | 0.49 |
| One week later, | 34 | 3,860,000 | 0.44 |

Four days after the temperature had subsided and one week

# CHART VII.

CASE 7. M.G., AET. 1 1/2 YEARS. NUTRITIONAL ANAEMIA.

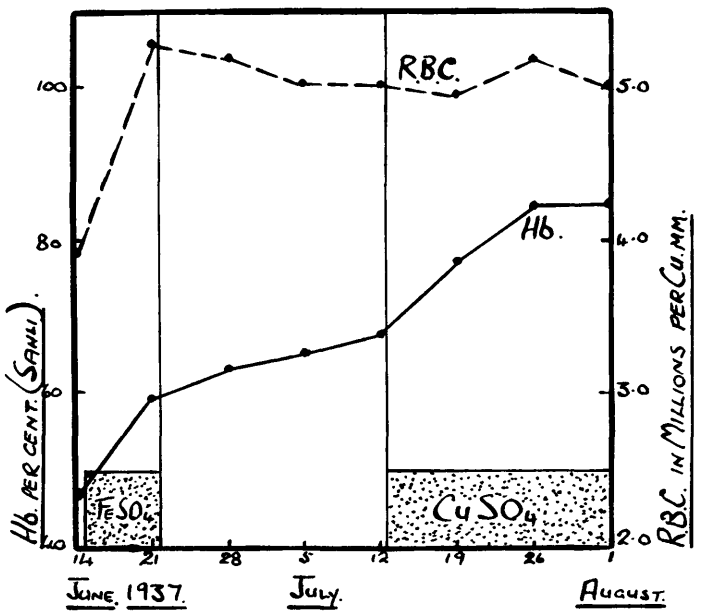
TREATED BY LARGE DOSES OF IRON FOLLOWED BY COPPER.



after the transfusion, ferrous sulphate was commenced in doses of 8 grammes per week and continued for two weeks. An iron-balance study was conducted during the two weeks of iron therapy, and for two weeks after discontinuance of iron administration, by which time there was a positive iron-balance (Table IX). It will be seen (Chart VII) that during the two weeks of iron therapy and the week immediately following, the haemoglobin level rose from 34 per cent to 77 per cent. Throughout the next three weeks the haemoglobin rose from 77 only to 82 per cent. At this time copper was added to the diet as 40 milligrammes of cupric sulphate per week, and in this next week the haemoglobin rose from 82 per cent to 95 per cent. Considered in terms of the iron added to the circulation, the effect of copper is more clearly demonstrated (Table IX). During the four weeks of the metabolism study 1,493.17 milligrames of iron were retained in the body. Of this amount, during the two weeks of iron therapy and the week immediately following (which is included because of the "lag effect" of the iron recently ingested) 87 milligrammes were utilized in haemoglobin synthesis, i.e. an average of 29 milligrammes per week. During the next three weeks, although there were 1,400 milligrammes of iron stored in the body, only 11 milligrammes went to the formation

# CHART VIII.

CASE 8. M.M^c C., AET. 1 5/12 YEARS. AMENTIA AND NUTRITIONAL ANAEMIA.
TREATED BY LARGE DOSES OF IRON FOLLOWED BY COPPER.



of haemoglobin, an average of 3.67 milligrammes per week. After the exhibition of copper, however, 26 milligrammes of iron were utilized in the next week.

Case 8. M.McC., aged one year, seven months. Amentia and Nutritional Anaemia.

This girl was admitted because of mental deficiency and nutritional anaemia. As in Case 7, treatment consisted first of iron and then of copper supplements to a diet of low iron-content. The dose of iron was 4 grammes of ferrous sulphate over a period of one week. Three weeks later she was given 40 milligrammes of copper sulphate for a further three weeks. An iron metabolism study was made from the commencement of treatment until two weeks after the cessation of iron therapy (Table X). The haemoglobin rose from the initial level of 47 per cent., before treatment was instituted, to 63 per cent one week after the end of iron administration (Chart VIII). In the ensuing two weeks a further rise of only 4 per cent occurred, but following the addition of copper at this time, the haemoglobin level rose in the next two weeks, from 67 per cent to 85 per cent, i.e. at the rate of 1.3 per cent per diem. If now, the iron equivalents of these haemoglobin values are considered in relation to the total iron retention in the body (Table X) of

401.62 milligrammes, as a result of the administration of 803.45 milligrammes of iron by mouth, it is found that during the week of iron therapy and the subsequent week, 44 milligrammes of iron (i.e. 10.9 per cent of the total retention) were added to the circulation. During the next two weeks only 10 milligrammes of iron (i.e. 2.5 per cent of the total retention) were utilized in haemoglobin building, but during the next two weeks following the start of copper therapy, the total haemoglobin iron rose by another 49 milligrammes (i.e. 12.2 per cent of the total retention).

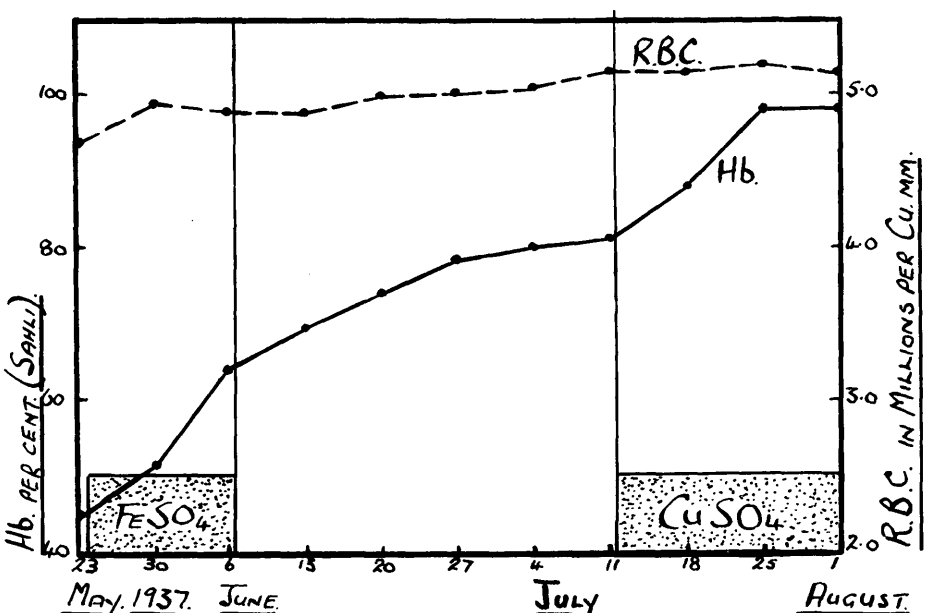
Case 9. J.M., aged one year. Nutritional Anaemia.

This patient was referred to hospital because of pallor. Examination of the blood showed a haemoglobin level of only 45 per cent. Four grammes of ferrous sulphate per week were given for two weeks. During this period and over the subsequent two weeks the iron balance was estimated. Three weeks after completion of the metabolism study, i.e. five weeks after the iron was discontinued, 40 milligrammes of copper sulphate per week were given for three weeks. The effect of treatment on the haemoglobin level is shown in Chart IX and on the iron balance in Table XI. The rise in the haemoglobin level from 45 per cent to 69 per cent one week after the end of iron

# CHART IX.

CASE 9. J. M., AET. 1 YEAR. NUTRITIONAL ANAEMIA.

TREATED BY LARGE DOSES OF IRON FOLLOWED BY COPPER.



treatment represented the addition of 73 milligrammes of iron to an 0.530 litre blood volume (24.3 milligrammes per week). The subsequent rise of 12 per cent in the next four weeks to a haemoglobin level of 81 per cent was equivalent to an increase in haemoglobin iron at the rate of 9.5 milligrammes per week. The administration of copper, however, was accompanied by a further increase of 54 milligrammes in the haemoglobin iron in two weeks, i.e. 26.5 milligrammes per week, which had raised the haemoglobin level from 81 to 95 per cent. This figure is 10 per cent above the "normal" level for this age (Mackay 1933)[2]. Furthermore, it is interesting to note that of the 732.61 milligrammes of iron retained by this infant as a result of iron treatment, only 22.4 per cent was utilized in the formation of new haemoglobin, and 7.2 per cent of the total retention was liberated for haemoglobin synthesis by copper.

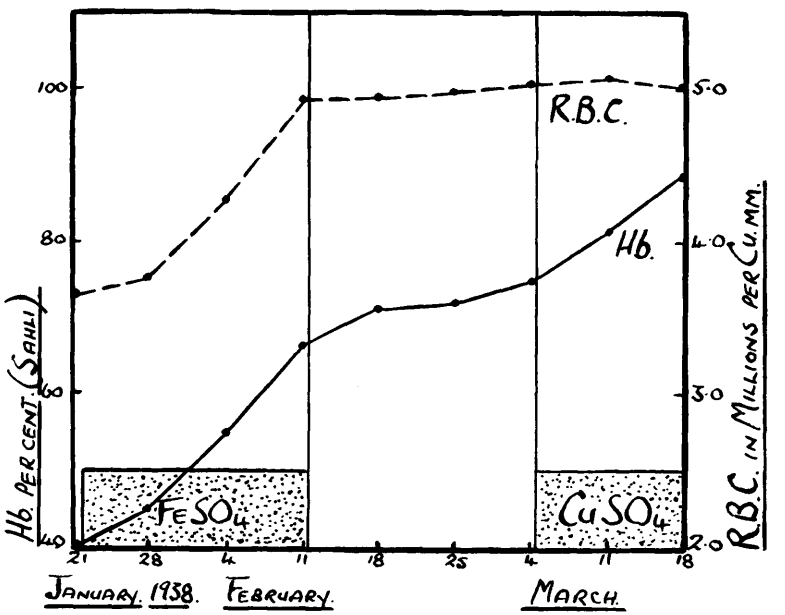
Case 10. H.C., aged three years three months. Nutritional Anaemia.

Admitted to hospital because of failure to thrive and found to have a nutritional anaemia. The patient was placed on a diet of adequate caloric value with an iron-content of 23.31 milligrammes per week, and an iron metabolism study commenced immediately. One week

# CHART X.

CASE 10. H.C., AET. 3 1/2 YEARS. NUTRITIONAL ANAEMIA.

TREATED BY LARGE DOSES OF IRON FOLLOWED BY COPPER.



later the administration of ferrous sulphate, in doses which amounted to 4 grammes per week, was begun and continued for three weeks. Unfortunately the metabolism study was discontinued three weeks after the completion of the iron course, and before a positive iron balance was re-established (Table XII). This misfortune was due to the fact that the process of preparation of faeces and determination of its iron content is of necessity a lengthy one, with the result that it is about three weeks from the end of any one weekly period before the iron retention during that period can be determined. From Chart X it can be seen, that over the period of iron therapy, the haemoglobin level rose from 40 per cent to 66 per cent, i.e. 1.24 per cent per diem. During the next week the haemoglobin rose to 71 per cent due to a "lag effect" of the iron, but during the next two weeks there was a haemoglobin rise of only 4 per cent., i.e. 0.29 per cent per diem. Thereafter the child received 30 milligrammes of copper sulphate per week. During that period the haemoglobin level rose from 75 per cent to 88 per cent, i.e. 0.93 per cent per diem. As a result of the three weeks' course of iron this child retained approximately 530 milligrammes of iron (Table XII). Of this amount 115 milligrammes were utilized for haemoglobin

building during the iron course and the week thereafter, i.e. 28.75 milligrammes per week. During the next two weeks the haemoglobin increase represented an iron utilization of only 7.5 milligrammes per week. The rise in the haemoglobin level from 75 to 88 per cent in the two weeks following the use of copper, however, represented the appropriation by the haemopoietic system of 46 milligrammes of iron, i.e. 23 milligrammes per week.

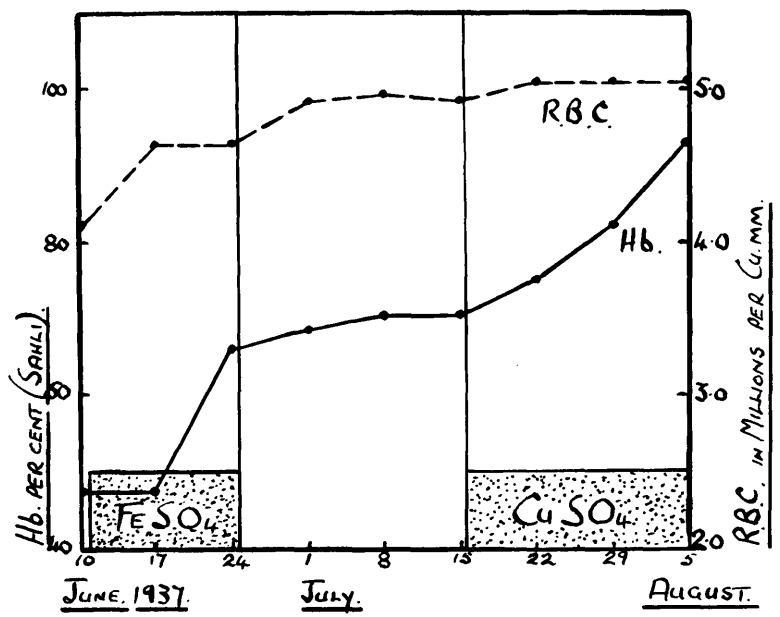
Case 11. R. O'D., aged ten months. Nutritional Anaemia and Rickets.

No vitamin D was administered until cure of the anaemia had been obtained with iron and copper in order that the haemoglobin response would be uninfluenced by other factors. An iron balance study was instituted after 4 days on a fixed diet, and at the end of the first week treatment started with 4 grammes of ferrous sulphate per week, for two weeks. The iron was then omitted and three weeks later a three-week course of copper (40 milligrammes per week) was given. As a result of the iron therapy 719.57 milligrammes of iron were stored in the body (Table XIII). Sixty-three milligrammes of this amount were utilized for haemoglobin synthesis during the two weeks of iron administration (31.5 milligrammes per week) to produce a haemoglobin rise of 18 per cent (1.29 per cent per diem),

# CHART XI.

CASE 11. R.O'D., AET. 10½ YEARS. NUTRITIONAL ANAEMIA AND RICKETS.

TREATED BY LARGE DOSES OF IRON FOLLOWED BY COPPER.



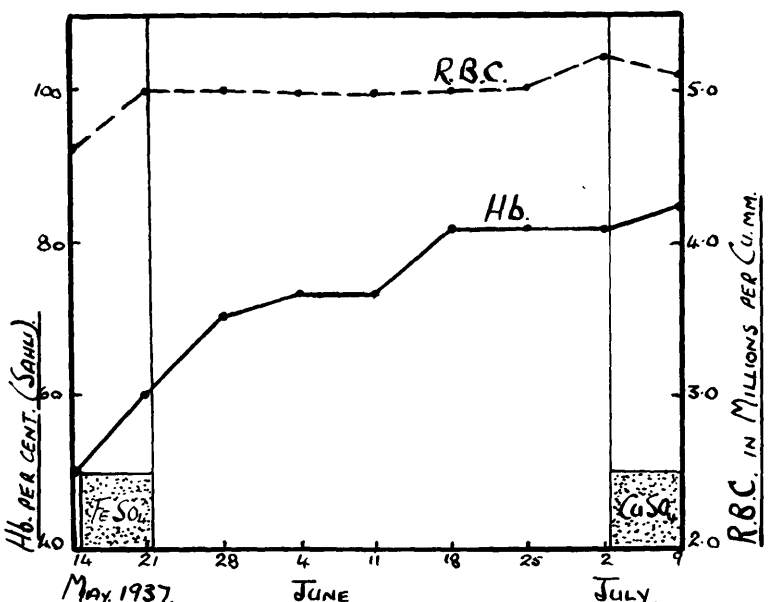
(Chart XI). During the next three weeks there was a rise of only 4 per cent in the haemoglobin level, representing the utilization of 15 milligrammes of iron (5.0 milligrammes per week). Following the use of copper during the ensuing three weeks, the haemoglobin rose from 70 to 93 per cent (1.10 per cent per diem), necessitating the liberation of 79 milligrammes of iron from the storage depots, i.e. at the rate of 26.3 milligrammes per week. Thus 157 milligrammes of iron transported from the storage depots to the bone marrow for utilization in haemoglobin synthesis, over 50 per cent was liberated as the result of copper action.

Case 12. M.G., aged one year, ten months. Nutritional Anaemia.

This girl, the twin of M.G. (Case 7) presented a much less profound degree of nutritional anaemia and in contrast to her sister was moderately well nourished. The mother insisted that the dietetic regime had been the same for both children from birth, and there had been no previous illnesses to explain the difference in their physical condition. As will be seen from Table XIV and Chart XII this girl responded to treatment very differently from her twin. Eight grammes of ferrous sulphate were given over a period of seven days with

## CHART XII.

CASE 12. M.G., AET. 1 11/12 YEARS. NUTRITIONAL ANAEMIA TREATED BY LARGE DOSES OF IRON FOLLOWED BY COPPER.



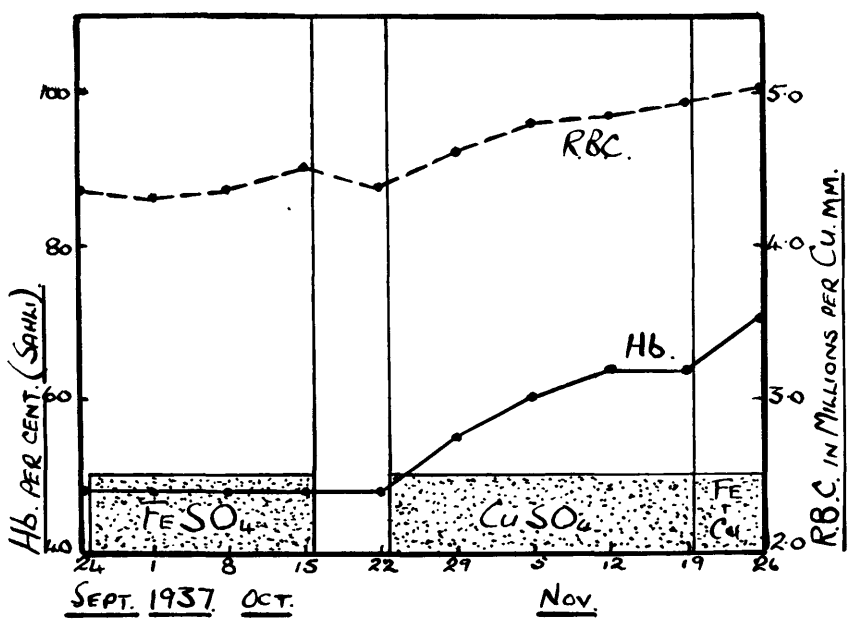
retention of 589.11 milligrammes of iron. Thereafter no further iron was given. This was followed by a haemoglobin response from 50 per cent to 73 per cent. One week later, however, the haemoglobin level rose from 73 per cent to 82 per cent (1.29 per cent per diem) in a period of seven days. A most careful inquiry into the dietetic and nursing regime failed to find an explanation for this occurrence. The haemoglobin level showed no further tendency to rise until the administration of 40 milligrammes of copper sulphate two weeks later, which caused a further rise of 3 per cent during the next week.

Case 13. H.C., aged one year four months. Nutritional Anaemia.

Admitted with severe nutritional anaemia:- haemoglobin 48 per cent, red-blood cells 4,360,000 per c.mm.. The administration of ferrous sulphate was started in doses of 0.5 gramme per week, and continued for three weeks. An iron-balance study during this period (Table XV) showed that the body retained 76.39 milligrammes of iron, and that a positive iron balance was present during the week immediately following the discontinuance of iron administration. The presence of this amount of iron in the body was not associated with any rise in the haemoglobin

## CHART XIII.

CASE 13. H.C., AET. 1½ YEARS. NUTRITIONAL ANAEMIA.
TREATED BY SMALL DOSES OF IRON FOLLOWED BY COPPER.



level (Chart XIII). One week after discontinuance of iron administration, cupric sulphate was given in doses amounting to 40 milligrammes per week, and three weeks later the haemoglobin level had risen by 15 per cent to 63 per cent, a rise which necessitated the addition to the circulation of 56 milligrammes of iron, i.e. 73 per cent of the total retention of 76.39 milligrammes. No further rise in haemoglobin took place until the administration of iron in therapeutic doses caused a further rise to commence, the haemoglobin level ultimately reaching a normal level.

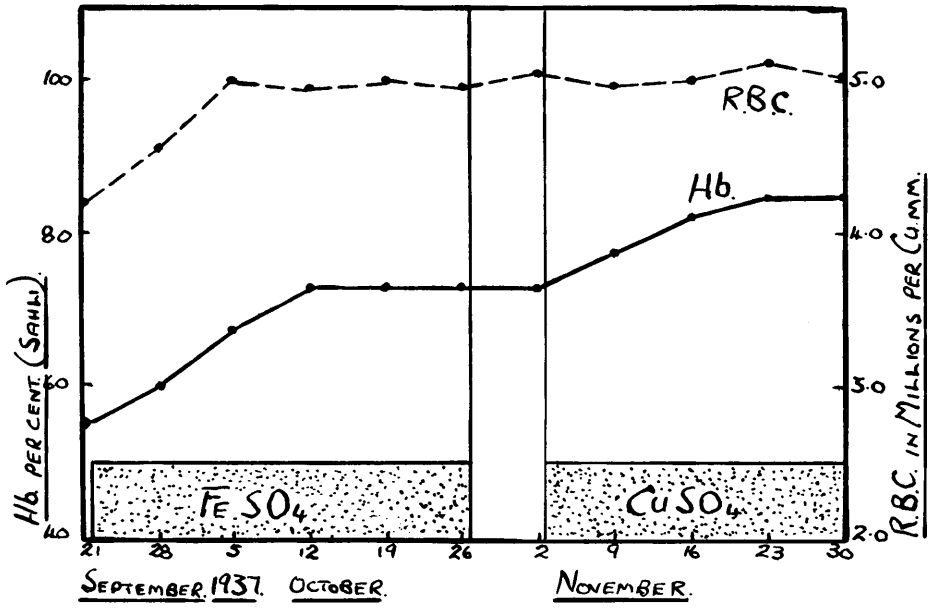
Case 14. M. McL., aged one year, three months. Nutritional Anaemia.

Ferrous sulphate (0.5 gramme per week) was administered to this case of nutritional anaemia (haemoglobin 55 per cent) over a period of five weeks. The metabolism study was unfortunately stopped, probably one week before the iron balance would have become positive, following the discontinuance of iron administration (Table XVI). The difficulty of judging as to when to stop the collection and preparation of faeces was noted in Case 10. In contrast to the progress of events in the previous patient, this dosage of iron caused the haemoglobin level to rise from 55 per cent to 72 per

# CHART XIV.

CASE 14. M.M.L., AET. 1 3/12 YEARS. NUTRITIONAL ANAEMIA.

TREATED BY SMALL DOSES OF IRON FOLLOWED BY COPPER.



cent in three weeks (Chart XIV) equivalent to the addition of 57 milligrammes of iron to the blood (Table XVI). Three weeks later, however, the haemoglobin level remained at 72 per cent, althoguh there were still present in the body 57.45 milligrammes of the total retention of 114.45 milligrammes of iron available for conversion into haemoglobin. Following the exhibition of copper at this time the haemoglobin level rose to 85 per cent in the next three weeks (0.62 per cent per diem) involving the utilization of 74 per cent (42 milligrammes) of the 57.45 milligrammes of iron probably still stored in the body. It is of interest to note here, that it is recognised to be more difficult to obtain a rise in the haemoglobin level when it approaches normal, than to effect the initial rise from the low level of the untreated anaemia. In this case the rise from 72 to 85 per cent was effected by the administration of copper alone, after the cessation of a minimal iron dosage. The ability of copper to produce a high final haemoglobin level in some instances has been a notable feature in this series of cases.

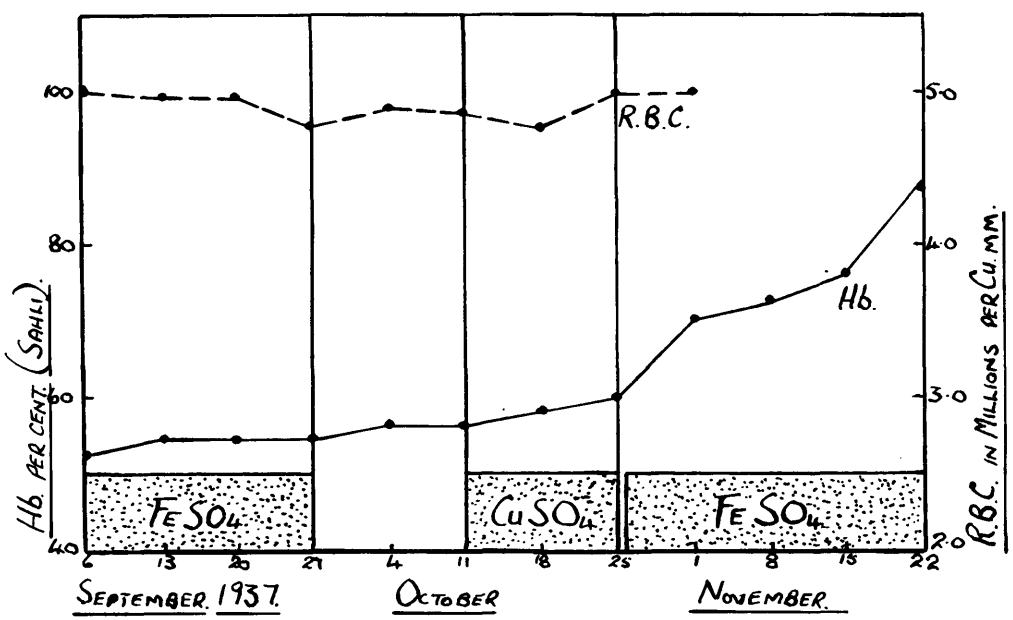
Case 15. J. McF., aged one year,two months. Nutritional Anaemia.

In this patient, admitted with a fairly severe nutri-

## CHART XV.

CASE 15. J. Mc F., AET. 1 2/12 YEARS. NUTRITIONAL ANAEMIA.

TREATED BY SMALL DOSES OF IRON FOLLOWED BY COPPER.



tional anaemia, copper failed to produce the desired satisfactory result. Ferrous sulphate was given, in doses amounting to 0.5 gramme per week for three weeks. An iron-balance study of five weeks' duration (Table XVII) revealed that the body had stored 58.18 milligrammes of iron as a result of iron administration, of which amount, 13 milligrammes were utilized to produce a rise in the haemoglobin level from 52 to 56 per cent (Chart XV). The administration of copper at this time was followed by the very small rise of 4 per cent haemoglobin in the next two weeks although there were still stored in the body 32.18 milligrammes of iron. Following the administration of therapeutic doses of iron, at this time, the haemoglobin rose to 88 per cent in four weeks. The irregularity of the response to this final iron therapy was, in part no doubt, due to the fact that this phase of the treatment was conducted at home, and coincided with the appearance of convulsions of unknown aetiology which the mother attributed to the medicine, the result being that the iron was not given with the desired regularity, nor in the prescribed amounts.

Discussion.

The foregoing results confirm the previous finding (Part II) that the body is capable of retaining large amounts of

iron administered in the inorganic state, and that in anaemia only a small percentage of the total retention is available for haemoglobin synthesis. This is in agreement with the findings of other workers (Brock, 1937; Fowler and Barer, 1935, 1937; Widdowson and McCance, 1937). They further show that, on discontinuance of iron administration, although there is enough iron in the body to provide for several times the haemoglobin requirements, further utilization of this iron for those requirements does not take place to any marked extent. In Case 7, although after the haemoglobin level had risen from 34 to 77 per cent, there were still 1,400 milligrammes of iron available in the body, the haemoglobin level rose only a further 5 per cent during the next three weeks. The administration of copper, however, caused an increase in the haemoglobin level of 13 per cent in seven days. Similar results were obtained in Cases 8, 9, 10, and 11. Even in Case 12, although the haemoglobin response was for some reason inconsistent, copper-produced a slight rise after the level had remained stationary for two weeks. A study of the results obtained in Cases 13, 14 and 15 reveals that the inefficacy of small doses of iron (in contrast to the massive therapeutic doses) does not depend upon imperfect absorption from the gut. Thus in Case 13, although the small doses of iron failed to produce any change in the

haemoglobin level, they did result in the retention of 76.39 milligrammes of iron, none of which was utilized for haemoglobin synthesis until the administration of copper was begun. These three cases therefore confirm the previous finding (Part II) that the necessity for large doses of iron does not lie in their ability to raise the iron content of the intestine above a certain "threshold value", presumed to exist by some previous workers. In view of these results there can be little doubt that small amounts of copper are capable of causing a considerable increase in the rate of haemoglobin formation.

Regarding the mode of action of copper, as this element does not form part of the haemoglobin molecule (Elvehjem, Steenbock, and Hart, 1929), it does not combine with iron directly in forming haemoglobin. It would appear, therefore, to have a catalytic action, either by liberating more iron from the liver for the use of the bone-marrow (Muntwyler and Hanzal, 1933) or by converting iron, which is stored in the tissues, into a chemical form more suited for inclusion in the haemoglobin molecule (Elvehjem and Sherman, 1932; Cunningham, 1931).

A suggestion which does not clash with the facts already known, based on the preceding observations, is put forward. That large amounts of iron are necessary to cure

anaemia is well recognised (Heath, 1933; Witts, 1933), but it is not as yet fully understood why this should be the case. An explanation advanced in Part II of this thesis was to the effect that iron once absorbed and stored in the liver was no longer available for haemoglobin formation; and the suggestion was made that massive doses were required to overcome the rate at which iron could be stored in the liver, with the result that some iron would overflow into the blood-serum and thus be transported in an available form to the bone-marrow. Furthermore, it was noted that there was apparently little tendency for iron, once stored in the tissues, to be re-excreted. Other workers attacking this problem from different aspects have tended to confirm these conclusions. McCance and Widdowson (1937) have pointed out that, in some way, iron must be transported very efficiently about the body, and showed that there was little evidence to suggest that the body ever excreted unwanted iron, either in the urine or faces. Moore, Arrowsmith,and Quilligan (1937) have recently emphasized that there are two non-haemoglobinous forms of blood iron, (1) serum or plasma iron, (2) what they refer to as "easily-split off" blood iron, the physiological functions of which have not been established. Further work (Moore, Doan,and Arrowsmith, 1937) has led them to believe that the plasma or serum iron

is in fact iron which is being transported from the storage depots to the bone-marrow for utilization in haemoglobin formation. The chemical nature of serum iron is still obscure. That it is not in an inorganic state and is not dialyzable is certain. It is probably trivalent and in organic combination, possibly as a complex ion (Moore, Doan, and Arrowsmith, 1937).

Applying these facts to the present series, it is possible to advance a hypothesis regarding the mode of copper action which is in line with previous work. The administration of iron produced a large iron-storage in the body. Of this store only a small amount went to haemoglobin formation, the remainder being apparently unavailable as the haemoglobin level ceased to rise appreciably ( save in Case 12), until the administration of copper caused some of the stored iron to be liberated into the blood-stream for transportation to, and utilization by, the haematopoietic centres in the bone-marrow. Whether copper acts, as Cunningham (1931) suggests, by a preliminary formation in the liver of a copper porphyrin and by its subsequent replacement by iron must remain a matter for further research. There would appear to be little doubt, however, that copper plays an active part in the genesis of haemoglobin. If it is accepted that copper is not contained in the haemoglobin

molecule, its action must be in the nature of a catalyst, and as iron is probably mainly stored in the liver, it probably acts in that situation.

It would, therefore, seem wise in the case of the iron deficiency anaemias of infancy and childhood at any rate, to include some copper in all iron prescriptions. This would ensure that there was a sufficiency of serum iron to meet the needs of the bone-marrow, by liberating iron from the liver into the blood-serum and would act as a supplement to that iron, which escaping the storage depots of the liver had already overflowed into the systemic circulation. Thus would a maximum and most rapid haemoglobin response be obtained in every case. This would be of advantage because of the marked susceptibility of those infants to infection, during which the administration of iron is ineffective (Mackay, 1931; Minot and Heath, 1932).

Summary.

1. The literature regarding the role of copper in the iron-deificiency anaemias is reviewed.

2. Metabolism studies on nine infants revealed (a) that the administration of copper enhances the conversion of iron stored in the tissue into haemoglobin, (b) that iron given in doses so small as not materially to raise the haemoglobin content of the blood can, by subsequently giving copper, be

mobilized and converted into haemoglobin.

3. It is suggested that copper acting as a catalytic agent enables iron to be converted into such a form that it can be transported by the blood-plasma from the storage depots to the bone-marrow whereit can be utilized in the formation of haemoglobin.

## TABLE IX.

Showing the Action of Copper in the Treatment of Anaemia with Large Doses of Iron.

| Week of Treatment. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe | Hb %. | R.B.C. per cu. mm. | Iron (Mgm.) as Hb. | Iron (Mgm.) Retained as Hb. |
|---|---|---|---|---|---|---|---|
| 1 | | | | 34 | 3,860,000 | 70 | |
| 2 | 1629.69 | 513.58 | 1116.21 | 50 | 4,360,000 | 103 | 33 |
| 3 | 1635.04 | 1221.11 | 413.93 | 68 | 4,240,000 | 140 | 37 |
| 4 | 28.14 | 70.49 | -42.53 | 77 | 5,000,000 | 157 | 17 |
| 5 | 28.14 | 22.76 | 5.38 | 79 | 4,960,000 | 162 | 5 |
| 6 | | | | 80 | 5,200,000 | 163 | 1 |
| 7 | 40 mgm. $CuSO_4$ per week. | | | 82 | 5,200,000 | 168 | 5 |
| 8 | | | | 95 | 5,200,000 | 194 | 26 |
| 9 | | | | 95 | 5,200,000 | 194 | Nil. |

Case 7. M.G., eat. 1,10/12 years. Nutritional Anaemia.

Weight 5.30 Kilos. Blood Volume - $\frac{5.30}{15}$ - 0.353 litres.

## TABLE X.

### Showing the Action of Copper in the Treatment of Anaemia with Large Doses of Iron.

| Week of Treatment. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | Hb %. | R.B.C. per cu. mm. | Iron (Mgm.) as Hb. | Iron (Mgm.) Retained as Hb. |
|---|---|---|---|---|---|---|---|
| 1 | | | | 47 | 3,960,000 | 127 | |
| 2 | 822.77 | 369.66 | 453.11 | 59 | 5,280,000 | 160 | 33 |
| 3 | 19.32 | 85.92 | -66.60 | 63 | 5,200,000 | 171 | 11 |
| 4 | 19.32 | 4.21 | 15.11 | 65 | 5,040,000 | 175 | 4 |
| 5 | 40 Mgm. $CuSO_4$ per week. | | | 67 | 5,040,000 | 181 | 6 |
| 6 | | | | 77 | 4,960,000 | 208 | 27 |
| 7 | | | | 85 | 5,200,000 | 230 | 22 |
| 8 | | | | 85 | 5,020,000 | 230 | Nil |

Case 8. M.McC., aet. 1,7/12 years. Nutritional Anaemia and Amentia.

Weight 7.00 Kilos. Blood Volume - $\frac{7.00}{15}$ - 0.467 litres.

## TABLE XI.

### Showing the Action of Copper in the Treatment of Anaemia with Large Doses of Iron.

| Week of Treatment. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | Hb %. | R.B.C. per cu. mm. | Iron (Mgm.) as Hb. | Iron (Mgm.) Retained as Hb. |
|---|---|---|---|---|---|---|---|
| 1 | | | | 45 | 4,710,000 | 138 | |
| 2 | 831.59 | 431.09 | 400.50 | 51 | 4,960,000 | 156 | 18 |
| 3 | 831.59 | 439.61 | 391.98 | 64 | 4,880,000 | 197 | 41 |
| 4 | 28.14 | 88.01 | -59.87 | 69 | 4,840,000 | 211 | 14 |
| 5 | 28.14 | 28.14 | Nil | 74 | 5,000,000 | 227 | 16 |
| 6 | | | | 78 | 5,020,000 | 239 | 12 |
| 7 | | | | 80 | 5,120,000 | 245 | 6 |
| 8 | 40 Mgm. $CuSO_4$ per week. | | | 81 | 5,200,000 | 249 | 4 |
| 9 | | | | 88 | 5,200,000 | 270 | 21 |
| 10 | | | | 98 | 5,240,000 | 302 | 32 |
| 11 | | | | 98 | 5,124,000 | 302 | Nil. |

Case 9. J.M., aet. 1 year. Nutritional Anaemia.

Weight 7.93 Kilos. Blood Volume - $\frac{7.93}{15}$ - 0.53 litres.

## TABLE XII.

### Showing the Action of Copper in the Treatment of Anaemia with Large Doses of Iron.

| Week of Treatment. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | Hb %. | R.B.C. per cu. mm. | Iron (Mgm.) as Hb. | Iron (Mgm.) Retained as Hb. |
|---|---|---|---|---|---|---|---|
| 1 | 23.31 | 12.39 | 10.92 | 40 | 3,630,000 | 146 | |
| 2 | 832.01 | 589.72 | 242.72 | 45 | 3,760,000 | 165 | 19 |
| 3 | 832.01 | 420.72 | 411.29 | 55 | 4,310,000 | 201 | 36 |
| 4 | 832.01 | 635.43 | 196.58 | 66 | 4,930,000 | 242 | 41 |
| 5 | 28.56 | 289.56 | -261.00 | 71 | 4,950,000 | 261 | 19 |
| 6 | 28.56 | 57.09 | -28.53 | 72 | 5,050,000 | 265 | 4 |
| 7 | 28.56 | 51.06 | -22.50 | 75 | 5,100,000 | 276 | 11 |
| | 30 Mgm. $CuSO_4$ per week. | | | | | | |
| 8 | | | | 81 | 5,140,000 | 297 | 21 |
| 9 | | | | 88 | 5,030,000 | 322 | 25 |

Case 10. H.C., aet. 3,3/12 years. Nutritional Anaemia.

Weight 9.49 Kilos. Blood Volume - $\frac{9.49}{15}$ - 0.633 litres.

## TABLE XIII.

### Showing the Action of Copper in the Treatment of Anaemia with Large Doses of Iron.

| Week of Treatment. | Intake Mgm. Fe | Output Mgm. Fe. | Retention Mgm. Fe. | Hb %. | R.B.C. per cu. mm. | Iron (Mgm.) as Hb. | Iron (Mgm.) Retained as Hb. |
|---|---|---|---|---|---|---|---|
| 1 | 20.37 | 13.21 | 7.16 | 48 | 4,120,000 | 170 | |
| 2 | 823.82 | 374.66 | 449.16 | 48 | 4,660,000 | 170 | Nil. |
| 3 | 823.82 | 514.31 | 309.51 | 66 | 4,660,000 | 233 | 63 |
| 4 | 20.37 | 59.37 | -39.00 | 68 | 4,960,000 | 242 | 9 |
| 5 | 20.37 | 20.47 | -0.10 | 70 | 4,980,000 | 248 | 6 |
| 6 | 40 Mgm. $CuSO_4$ per week. | | | 70 | 4,960,000 | 248 | 0 |
| 7 | | | | 75 | 5,040,000 | 266 | 18 |
| 8 | | | | 82 | 5,100,000 | 291 | 25 |
| 9 | | | | 93 | 5,080,000 | 327 | 36 |

Case 11. R.O'D., aet. 10/12 years. Nutritional Anaemia and Rickets.

Weight 9.17 Kilos. Blood Volume - $\frac{9.17}{15}$ - 0.611 litres.

## TABLE XIV.

### Showing the Action of Copper in the Treatment of Anaemia with Large Doses of Iron.

| Week of Treatment. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | Hb %. | R.B.C. per cu. mm. | Iron (Mgm.) as Hb. | Iron (Mgm.) Retained as Hb. |
|---|---|---|---|---|---|---|---|
| 1 | | | | 50 | 4,640,000 | 187 | |
| 2 | 1635.04 | 909.04 | 726.00 | 60 | 5,040,000 | 223 | 36 |
| 3 | 28.14 | 173.43 | -145.29 | 70 | 5,040,000 | 259 | 36 |
| 4 | 28.14 | 19.74 | 8.40 | 73 | 5,020,000 | 270 | 11 |
| 5 | | | | 73 | 5,000,000 | 270 | Nil |
| 6 | | | | 82 | 5,040,000 | 304 | 34 |
| 7 | | | | 82 | 5,080,000 | 304 | Nil |
| 8 | 40 Mgm. $CuSO_4$ per week. | | | 82 | 5,260,000 | 304 | Nil |
| 9 | | | | 85 | 5,080,000 | 315 | 11 |

Case 12. M.G., aet. 1,10/12 years. Nutritional Anaemia.

Weight 9.60 Kilos. Blood Volume - $\frac{9.60}{15}$ - 0.64 litres.

## TABLE XV.

### Showing the Action of Copper in the Treatment of Anaemia with Small Doses of Iron.

| Week of Treatment. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | Hb %. | R.B.C. per cu. mm. | Iron (Mgm.) as Hb. | Iron (Mgm. Retained as Hb. |
|---|---|---|---|---|---|---|---|
| 1 | | | | 48 | 4,360,000 | 181 | |
| 2 | 128.57 | 110.40 | 18.17 | 48 | 4,320,000 | 181 | Nil |
| 3 | 128.57 | 120.82 | 7.75 | 48 | 4,430,000 | 181 | Nil |
| 4 | 128.57 | 86.64 | 41.93 | 48 | 4,530,000 | 181 | Nil |
| 5 | 28.14 | 19.60 | 8.54 | 48 | 4,380,000 | 181 | Nil |
| | 40 Mgm. $CuSO_4$ per week. | | | | | | |
| 6 | | | | 55 | 4,640,000 | 205 | 24 |
| 7 | | | | 60 | 4,780,000 | 226 | 21 |
| 8 | | | | 63 | 4,800,000 | 237 | 11 |
| 9 | | | | 63 | 4,920,000 | 237 | Nil |
| | $FeSO_4$ gm.iv, $CuSO_4$ mgm.40 per week. | | | | | | |
| 10 | | | | 70 | 5,040,000 | 263 | 26 |

Case 13. H.C., aet. 1,4/12 years. Nutritional Anaemia.

Weight 9.72 Kilos. Blood Volume - $\frac{9.72}{15}$ - 0.65 litres.

TABLE XVI.

Showing the Action of Copper in the Treatment of Anaemia with Small Doses of Iron.

| Week of Treatment. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | Hb %. | R.B.C. per cu. mm. | Iron (Mgm.) as Hb. | Iron (Mgm.) Retained as Hb. |
|---|---|---|---|---|---|---|---|
| 1 | | | | 55 | 4,200,000 | 181 | |
| 2 | 128.57 | 51.01 | 77.56 | 60 | 4,600,000 | 198 | 17 |
| 3 | 128.57 | 107.43 | 21.14 | 67 | 5,020,000 | 222 | 24 |
| 4 | 128.57 | 90.71 | 37.86 | 72 | 4,980,000 | 238 | 16 |
| 5 | 128.57 | 148.64 | -20.07 | 72 | 5,020,000 | 238 | Nil |
| 6 | 128.57 | 117.58 | 10.99 | 72 | 4,960,000 | 238 | Nil |
| *7 | 12.06 | 25.09 | -13.03 | 72 | 5,040,000 | 238 | Nil |
| | 40 Mgm. $CuSO_4$ per week. | | | | | | |
| 8 | | | | 78 | 4,980,000 | 258 | 20 |
| 9 | | | | 82 | 5,020,000 | 271 | 13 |
| 10 | | | | 85 | 5,120,000 | 280 | 9 |
| 11 | | | | 85 | 5,020,000 | 280 | Nil |

* Only a three-day period due to error in disposal of faeces.

Case 14. M.McL., aet. 1,3/12 years. Nutritional Anaemia.

Weight 8.56 Kilos. Blood Volume - $\frac{8.56}{15}$ - 0.57 litres.

TABLE XVII.

Showing the Action of Copper in the Treatment of Anaemia with Small Doses of Iron.

| Week of Treatment. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | Hb %. | R.B.C. per cu. mm. | Iron (Mgm.) as Hb. | Iron (Mgm.) Retained as Hb. |
|---|---|---|---|---|---|---|---|
| 1 | | | | 52 | 5,020,000 | 169 | |
| 2 | 121.57 | 123.26 | -1.69 | 54 | 4,960,000 | 174 | 5 |
| 3 | 121.57 | 58.97 | 62.60 | 54 | 4,960,000 | 174 | Nil |
| 4 | 121.57 | 108.98 | 12.59 | 54 | 4,720,000 | 174 | Nil |
| 5 | 21.14 | 45.10 | -23.96 | 56 | 4,920,000 | 182 | 8 |
| 6 | 21.14 | 12.50 | 8.64 | 56 | 4,800,000 | 182 | Nil |
| | 40 Mgm. $CuSO_4$ per week. | | | | | | |
| 7 | | | | 58 | 4,760,000 | 188 | 6 |
| 8 | | | | 60 | 5,020,000 | 195 | 7 |
| | $FeSO_4$ gm.iv per week. | | | | | | |
| 9 | | | | 70 | 5,040,000 | 227 | 32 |
| 10 | | | | 72 | ------- | 235 | 8 |
| 11 | | | | 76 | ------- | 246 | 11 |
| 12 | | | | 88 | ------- | 285 | 39 |

Case 15. J.McF., aet. 1,2/12 years. Nutritional Anaemia.

Weight 8.44 Kilos. Blood Volume - $\frac{8.44}{15}$ - 0.56 litres.

## PART IV. The Influence of Infection on the Haematopoietic Tissues.

It has already been pointed out that an infective process may be an important factor in producing an anaemia (Part I). Hypochromic anaemia is commonly found in sepsis, syphilis, nephritis, the cachexia of malignant disease, etc. (Collins, 1935; Sturgis, Isaacs, Goldhammer, Bethel, and Farrar, 1935; Vaughan, 1936). In these and many other diseases the haematopoietic system is apparently unable to utilize iron or other substances, even though these are administered in massive doses. The anaemia is probably due to a number of causes, as yet ill-defined, of which deficiency of an essential nutritional factor is not the most important. A better knowledge of the pathogenesis of this type of anaemia would probably also throw light on the cause of the diminished response to iron and liver therapy in cases of iron-deficiency and pernicious anaemia when complicated by infections, a well recognised fact as yet awaiting explanation (Paxton, 1936; Beebe and Lewis, 1931; Minot and Heath, 1932; Parsons, 1931). In some infections, e.g. those due to the haemolytic streptococcus, B.welchii, and Bartonella bacilliformis, haemolysins are produced in the blood-stream (Vaughan, 1936); in other

rare instances of profound toxaemia the anaemia may be due, in part, to a severe symptomatic purpura (Kugelmass and Lampe, 1932). In the majority of infections such factors are absent, and a careful perusal of the literature has failed to reveal much in the way of systematic studies of the anaemia associated with infection. Davidson and Fullerton (1938) state that toxic retention in general depresses the function of the bone-marrow and produces a blood picture characteristic of a "semi-aplastic" anaemia in which the blood level falls with little change in the appearance of the erythrocytes. Kugelmass and Lampe (1932) found thepresence of a hypochromic anaemia in most cases of pneumonia, otitis media, tuberculosis, and chronic nephritis in childhood; the presence in films of nucleated red cells was taken to indicate an increased blood-forming demand in such cases. In only a few cases was there any evidence of haemolysis. They concluded that myeloid insufficiency in the course of increased blood-forming demand results in anaemia, and that the retardation in myeloid function parallels the severity of the infection, being rapid in acute infections and slow but cumulative in chronic pyogenic infections. Smithburn, Masters and Zerfas (1930) noted that infection may be responsible for temporary or complete inhibition of the haemopoetic response during

treatment of "secondary anaemia" with iron salts. They attributed this as being due more probably to some deleterious effect on the haemopoietic system, than to actual destruction of the elements in the circulating blood. Robscheit-Robbins and Whipple (1936) studied the effect of infection and intoxication (e.g. sterile turpentine abscess) upon haemoglobin formation in the chronic post-haemorrhagic anaemia of dogs. Their experiments pointed to a disturbance of internal metabolism related to haemoglobin building, and negatived the possibility of impaired intestinal absorption or increased destruction of red cells in the blood. On the other hand, regarding the influence of infections on the nutritional anaemia of infancy, Josephs (1934) thought this to be productive of an iron deficiency, having demonstrated the presence of a negative iron-balance during a period of infection. However, the rapid fall in haemoglobin suggests to Fullerton (1937) that the toxins of infective processes cause, in addition, an inhibition of blood-formation. Heath and Patek (1937) state that complicating factors such as sepsis, nephritis, and cirrhosis of the liver inhibit the response of the haemaglobin and reticulocytes, but give no opinion as to how this effect is produced. Recent work suggests that at least in some infections, such as rheumatic fever, bone tuberculosis, and

Still's disease, there may be a deficiency of Vitamin C acting as a possible factor. Slight but definite reticulocyte responses have been noted following the administration of large doses of vitamin (Faulkner, 1935) in such cases. Markowitz (1932) put forward the opinion that such aplastic blood dyscrasies as "idiopathic" aplastic anaemia and agranulocytosis, were themselves an unusual response of the formative tissues to infection. The vague suggestion is made that some body constituent or modification of the normal defence reaction in the body interferes with the normal activation of the manufacture and destruction of cells of the haemopoietic tissues.

The possibility, that studies in the retention of massive doses of iron administered to anaemic and non-anaemic patients during a period of severe infection, might serve to elucidate this difficulty problem, has prompted the present investigation.

Method of Investigation.

Two cases of profound infection in association with almost normal blood pictures, and one case of nutritional anaemia complicated by acute infection, have been studied in the following manner. Ferrous sulphate was administered in doses amounting to 4 grammes (803.45 milligrammes of iron) per week, over prolonged periods. An iron-balance

study was made during the whole or part of the period of iron administration. As in the previous cases (Parts II and III) the diet of each patient was regulated for four days prior to the commencement of the metabolism study, and its iron content was known, and maintained at a low level. The blood picture was studied in each case by weekly determinations of the haemoglobin levels (using the standardised Sahli haemoglobinometer) and red cell counts. Other supplementary blood examinations directed towards the detection of excessive haemolysis were made when necessary.

In this way, and using the calculations already detailed (Parts II and III), it was possible to observe the effect of infection on (1) the iron content of the circulating haemoglobin, (2) the amount of iron rendered available to the body as the result of iron administration. The accurate assessment of these two factors allowed conclusions to be drawn regarding the unknown factor, viz., the ability of the formative tissues to utilize the iron introduced into the tissues by oral administration.

Results.

Case 16. A.M., aged one year, three months. Pneumococcal Empyema.

This infant was admitted with an empyema of the left side confirmed by radiographic examination and aspiration

of pus. On the 7th November 1937, the temperature was 102°F; pulse rate 160 per minute; respirations 45 per minute. Blood examination revealed a mild hypochromic anaemia; haemoglobin 76 per cent; red blood cells 4,360,000 per cu.mm.. Ferrous sulphate was started on the 8th November 1937, in doses of 4 grammes perweek, and continued for four weeks. The iron content of the diet was known (Table XVIII). The iron metabolism study had to be abandoned during the third week of observation due to mild diarrhoea. Throughout, the temperature varied from 100° to 103°F (per rectum), and pus was aspirated from the chest on five occasions. The total iron retention in the body over the six weeks of the metabolism study (at the rates shown in periods two, four and five) amounted approximately to 738 milligrammes, which was more than double the total amount of iron present in the circulating haemoglobin (approximately 308 milligrammes). (Table XVIII). In spite of this large amount of iron available in the body, the haemoglobin value did not show any tendency to rise from the initial level of 76 per cent; in fact rather did it tend to fall, reaching 72 per cent on one occasion. There was never any reticulocytosis or increase of urobilinogen in the

urine to indicate the presence of increased blood destruction. Nor was the ability of the intestine to absorb iron impaired to any great degree by the infection, as evidenced by the large iron retention and the institution of a positive iron-balance one week after discontinuance of iron administration. The patient made an uneventful recovery.

Case 17. T.G., aged seven years, eleven months. Lobar Pneumonia and Empyema.

This boy was admitted with a pneumonic consolidation of the right lower lobe.

On the 2nd November 1937, the temperature was 103°F.; pulse rate 140 per minute; respirations 40 per minute. The child was profoundly ill with marked delirium, and for some days his condition was critical. Blood examination showed: haemoglobin, 91 per cent; red blood cells, 4,960,000 per cu.mm. Ferrous sulphate was started in doses of 4 grammes per week on the 3rd November 1937, and continued well into the stage of convalescence; throughout, the blood was examined regularly (Chart XVI).

The child's condition slowly improved from about the 5th November 1937; the temperature, however, settled slowly by lysis and by the 16th November 1937, was

normal save for occasional small rises to 99°F.. At this time his condition was not quite satisfactory and the physical signs suggested fluid at the right base. On the 22nd November 1937, seventy cubic centimetres of pus were aspirated from the right pleural sac. Culture of the pus grew pneumococcus type I. Thereafter the pus was aspirated on three occasions, only very small amounts being obtained. The boy's general condition remained unsatisfactory, and from the 26th November 1937, the temperature again fluctuated between 102° and 103°F.. On the 18th December 1937, rib resection was performed and closed pleural drainage instituted. After the operation although very little pus was drained off, the boy's condition rapidly improved, and by the 4th January 1938, the wound had healed and the temperature remained normal. An iron metabolism study was commenced on the 3rd November 1937, and discontinued on the 15th December 1937, when the boy was transferred to the surgical wards for operation (Chart XVI). He was dismissed home on the 18th January 1938, still having ferrous sulphate and readmitted on the 8th February 1938, that the metabolism study might be completed. Administration of ferrous sulphate was stopped on 9th February 1938, (Table XIX), i.e. at the

end of the fifteenth week of observation.

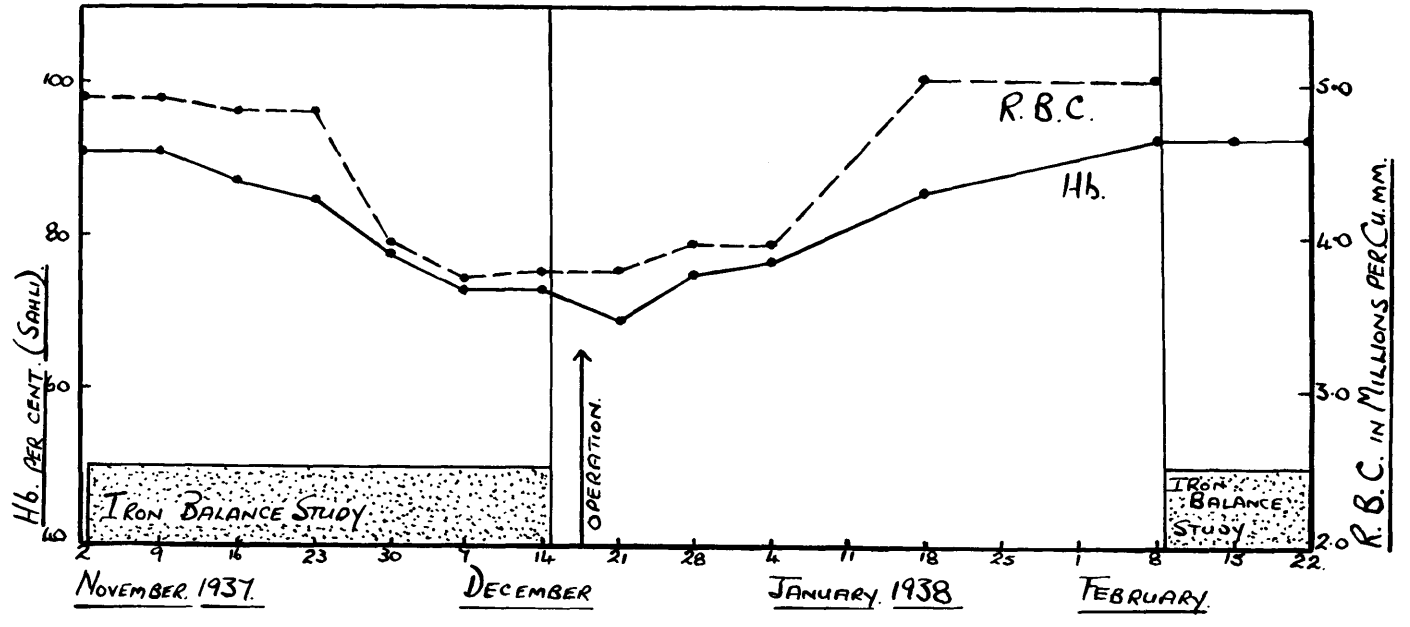
During the six weeks prior to operation, when the iron-balance was studied, and in the presence of severe infection and toxic absorption, the child retained 2048.96 milligrammes of iron in his tissues. This amount is approximately equivalent to the total amount of iron normally present in the whole body of a child of this age. At this rate of retention, the total iron retention in the body over the fourteen weeks of iron administration would be over 4.5 grammes. Furthermore, an iron-balance study, over a period of two weeks after discontinuance of iron administration, showed no appreciable tendency for any of this large retention to be excreted. The negative balance of 87.85 milligrammes probably represented unabsorbed iron left in the gut.

In spite of this large excess concentration of iron in the storage depots, the haemoglobin level fell steadily during the period of infection, from the initial level of 91 per cent to 72 per cent on the 7th December, 1937 (Chart XVI). There was no evidence of any appreciable degree of haemolysis; on the 30th November 1937, reticulocytes were 2 per cent, indirect Van den Bergh 1.0 unit, Schlesinger's test

## CHART. XVI.

CASE 17. T.G., AET. 7 11/12 YEARS. LOBAR PNEUMONIA AND EMPYEMA.

HAEMOGLOBIN DURING A PERIOD OF INFECTION.



for urobilinuria, negative. The subsequent fall in the haemoglobin level from 72 per cent to 69 per cent after the operation is probably attributable to the effects of the operation. Thereafter, with the improvement in the boy's condition the haemoglobin rose steadily to a maximum value of 92 per cent, a rise which was probably independent of the iron administration.

Case 18. D.F., aged one year, three months. Nutritional Anaemia and Bronchopneumonia.

On admission on the 5th March 1938, this child was found to have an acute pneumonic process involving the left upper lobe; there was a fever of 104°F., and the child was profoundly ill and toxaemic, although free from any severe dyspnoea or cyanosis. The fever settled by crisis on the 10th March 1938, and thereafter the child made a rapid and uneventful recovery. The acute short illness, absence of cough and fall of temperature by crisis are taken by McNeil and Macgregor et al. (1929) to indicate "alveolar" pneumonia, which they insist has the pathological features of lobar pneumonia, in contrast with the more common bronchopneumonia of this age-period.

Blood examination on the 7th March 1938 showed a

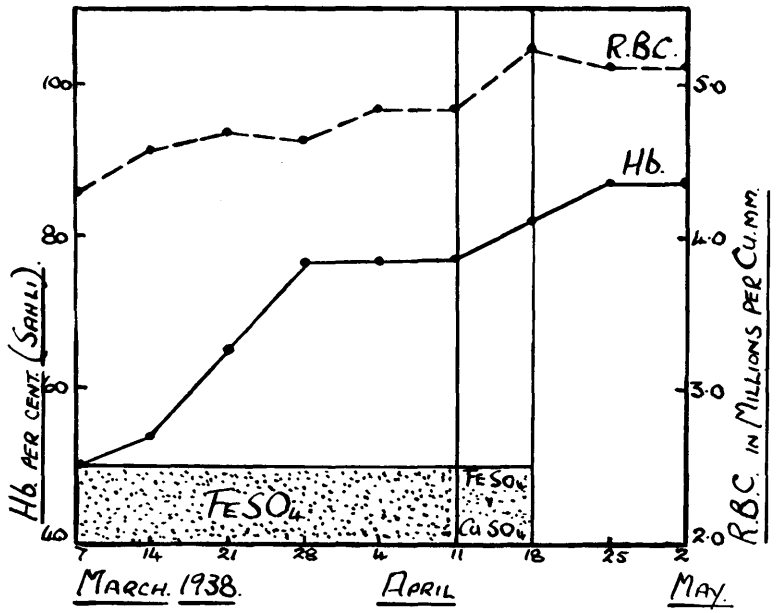
profound hypochromic anaemia:- haemoglobin 50 per cent, red blood cells 4,280,000 per cu.mm.; colour index 0.59. Treatment was started on the same day with 4 grammes of ferrous sulphate per week (Chart XVII). An iron metabolism study was commenced at this time, and continued until cure of the anaemia had been obtained (Table XX) as judged by weekly estimations of the haemoglobin and red cells. There was never any urobilinuria to indicate excessive haemolysis; the reticulocyte count before the institution of iron therapy was under 1 per cent.

It can be seen that during the 2nd period of observation (Table XX), i.e. the 1st week of the iron-balance study, during four days of which there was high fever and toxaemia, 51 per cent of the total iron intake was retained in the body. This figure is higher than that obtained during the 3rd and 7th weeks of observation when the child was no longer acutely ill. It would appear, therefore, that the toxaemia of the acute pneumonia was not associated with any impairment in the ability of the gut to absorb iron. At the same time, during this same period (7th to 14th March 1938) there was a haemoglobin rise of only 4 per cent as a result of iron therapy (Chart XVII) although the total iron

# CHART XVII.

CASE 18. D.F., AET. 1 3/12 YEARS. NUTRITIONAL ANAEMIA AND PNEUMONIA.

Hb. RESPONSE DURING A PERIOD OF INFECTION.



retention during the week (415.52 milligrammes) was enough to produce a haemoglobin level of over 100 per cent if fully utilised for that purpose. During the next two weeks when there was no fever, there were rises in the haemoglobin level of 11 per cent per week, to 76 per cent. Contrasting those changes in the haemoglobin level (expressed in terms of their iron equivalents), with the iron retentions at the same times, it is found (Table XX) that during the first period of the metabolism study, when there was fever, only 2.9 per cent of the total iron retention, during that week, was utilized for haemoglobin synthesis. During the next two periods when there was no fever, 11.1 per cent and 7.8 per cent of the total retentions for those periods were utilized for the formation of new haemoglobin.

When the haemoglobin level was 76 per cent, iron therapy alone failed to increase the level above 77 per cent (Chart XVII). The addition of 40 milligrammes of copper sulphate to the iron for one week caused the haemoglobin level to reach 87 per cent in the next fortnight. This finding would further confirm the conclusions drawn, regarding the value of copper as an adjunct to iron therapy, in Part III of this thesis.

Discussion.

It is recognised that apart from certain specific types, some of which have been instanced, the anaemia of infection is not associated with excessive haemolysis. The present results exclude impaired ability of the intestine to absorb iron as a factor, and on the basis of previous results (Part I), it should have been possible to maintain the haemoglobin values at high levels. The converse held, and in spite of large reserves of iron in the tissues (in Case 17) the haemoglobin values fell during a period of severe infection. In Case 18, it was seen that while fever did not impair the absorption of iron by the intestine, it did impair the capacity of the haematopoietic tissues to increase the haemoglobin level. The position is therefore, that although the necessary components of the cellular elements of the blood are present in sufficient quantity, there is a deficiency in the ability of the body to metabolise those components, due presumably to the action of some toxin or toxins on the haematopoietic tissues. Until a further understanding of the cellular metabolism of the formative tissues is at hand, it must remain impossible to state at what stage in the maturation of the erythrocyte inhibition occurs. Thus, it is not known whether the haemoglobin molecule is synthesised within

the early erythroblast, or whether the already synthesised molecule is added to the stroma of the cell from other sources. As in common with other workers (Weiss, 1931) the white blood cells were found to be increased in the present cases, the assumption can be made that the fault must lie with later forms of the developing erythrocyte than is the case in the so-called idiopathic aplastic anaemia, where the diminution commonly affects not only the red cells, but also the leucocytes and platelets. Even under this name, Turnbull (Vaughan, 1936) states that there are probably cases of different aetiology, having in common a loss of erythropoietic marrow, and he quotes the post-mortem findings in a case where the reduction of haemopoietic marrow had affected chiefly the erythropoietic elements, there being relatively little reduction in the number of megakaryocytes, myelocytes, and leucocytes. The anaemia of sepsis and infection differs from aplastic anaemia further in that the former is usually hypochromic, whereas in the latter the red cells are usually correspondingly reduced (Vaughan, 1936). In the present state of our knowledge, the term "hypoplastic anaemia" is suggested as an accurate description of the condition. This conception serves to impress the fact that iron therapy is likely to prove a failure in such types of anaemia; nor is there

any other erythroblast stimulating factor, analogous to "nucleotide K96" in agranulocytosis (Jackson et al., 1931). The only form of therapy available while the infection persists is blood transfusion, which, as in true primary aplastic anaemia, is only of temporary value, but serves in many instances to tide the patient over a critical period.

Summary.

1. Observations on the retention of iron have been made on two non-anaemic children and one anaemic child suffering from severe infections. There is no impairment in the ability of the body to absorb iron during a period of infection.

2. Infection inhibits the activity of the erythrogenic foci in the haematopoietic tissues, and this effect cannot be counteracted by the administration of massive doses of inorganic iron.

3. It is suggested that the anaemia of infection be designated "hypoplastic anaemia", and it is emphasised that the only effective form of treatment, apart from removal of the primary infection, is blood transfusion.

## TABLE XVIII.

### Absorption and Retention of Iron during a Period of Infection.

| Week of Observation. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | % Retention | Hb %. | R.B.C. per cu. mm. |
|---|---|---|---|---|---|---|
| 1 | | | | | 76 | 4,360,000 |
| 2 | 824.59 | 419.25 | 405.34 | 49 | 74 | 4,120,000 |
| 3 | 824.59 | ---------- | ---------- | ------ | 76 | 4,240,000 |
| 4 | 829.84 | 798.09 | 31.75 | 4 | 76 | 4,360,000 |
| 5 | 829.84 | 690.00 | 139.84 | 17 | 72 | 4,320,000 |
| 6 | 26.39 | 64.85 | -38.46 | Nil | 74 | 4,460,000 |
| 7 | 26.39 | 18.71 | 7.68 | 30 | 76 | 4,460,000 |

Case 16. A.M., aet. 1,3/12 years. Pneumococcal Empyema.

## TABLE XIX.

### Absorption and Retention of Iron During a Period of Infection.

| Week of Observation. | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | % Retention | Hb %. | R.B.C. per cu. mm. |
|---|---|---|---|---|---|---|
| 1 | | | | | 91 | 4,940,000 |
| 2 | 814.02 | 475.07 | 338.95 | 41 | 91 | 4,960,000 |
| 3 | 842.23 | 433.18 | 409.05 | 49 | 88 | 4,760,000 |
| 4 | 842.23 | 799.72 | 42.51 | 5 | 85 | 4,760,000 |
| 5 | 842.23 | 316.61 | 525.62 | 62 | 78 | 3,960,000 |
| 6 | 842.23 | 717.42 | 124.81 | 14 | 72 | 3,600,000 |
| 7 | 842.23 | 234.21 | 608.02 | 72 | 72 | 3,720,000 |
| 15 | - - - - - | - - - - - | - - - - - - | - - - - | 92 | 5,080,000 |
| 16 | 38.78 | 124.54 | -85.76 | Nil | 92 | - - - - - |
| 17 | 38.78 | 40.87 | -2.09 | Nil | 92 | - - - - - |

Case 17. T.G., aet. 7,11/12 years. Lobar Pneumonia and Empyema.

## TABLE XX.

### Retention and Utilisation of Iron in a Case of Nutritional Anaemia Complicated by Infection.

| Week of Observation | Intake Mgm. Fe. | Output Mgm. Fe. | Retention Mgm. Fe. | % Retention | Hb %. | R.B.C. per cu. mm. | Iron (mgm) as Hb | Iron(mgm) Retained as Hb. | % of Retention Utilised. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | 50 | 4,280,000 | 186 | | |
| 2 | 814.02 | 398.50 | 415.52 | 51 | 54 | 4,530,000 | 198 | 12 | 2.9 |
| 3 | 824.59 | 456.63 | 367.96 | 45 | 65 | 4,740,000 | 239 | 41 | 11.1 |
| 4 | 824.59 | 316.26 | 508.33 | 62 | 76 | 4,610,000 | 279 | 40 | 7.8 |
| 5 | 828.79 | - - - - - | - - - - - - | - - - | 77 | 4,780,000 | 283 | 4 | - - - |
| 6 | 828.79 | - - - - - | - - - - - - | - - - | 77 | 4,770,000 | 283 | Nil | Nil |
| 7 | 828.79 | 527.42 | 301.37 | 36 | 82 | 5,260,000 | 303 | 20 | 7.0 |
| 8 | 25.34 | 78.85 | -53.51 | Nil | 87 | 5,110,000 | 322 | 19 | Nil |
| 9 | 25.34 | 56.67 | -31.33 | Nil | 87 | 5,110,000 | 322 | Nil | Nil |

Case 18. D.F., aet. 1,3/12 years. Nutritional Anaemia and Pueumonia.

Weight 9.55 Kilos. Blood Volume - $\frac{9.55}{15}$ - 0.637 litres.

# Appendix.

## A Macromethod for the Determination of Iron in Biological Material by a Modified Permanganate Titration.

This method is designed for the estimation of iron in the presence of interfering material, e.g. organic matter and phosphates. The errors of micro-colorimetric methods are greatly exaggerated when amounts of iron between 1 and 10 milligrammes are estimated. Other existing methods for such amounts show many disadvantages. Destruction of organic matter by ignition is extremely time-consuming; on the other hand incineration with sulphuric acid was considered to present the advantages both of quickness and of removal of halogens which would interfere in the subsequent steps in the procedure.

The gravimetric method of estimation of iron requires either the removal of phosphate by molybdate, or the use of basic acetate separation. These modifications, together with the undesirability of using ammonia in the laboratory, ruled out this method of investigation. The use of a sulphuric acid digestion made possible the volumetric estimation of iron. The permanganate method of Margueritte (Treadwell and Hall, 1924) was chosen because of the

ease of determining the end-point and the fact that permanganate offers a self-contained indicator.

Reagents.

Sulphuric Acid: B.D.H. "Analar", low in heavy metals.

Perchloric Acid: B.D.H. 60 per cent perchloric acid, specific gravity 1.54, low in heavy metals.

Zinc: McCulloch's 20 mesh granular, low in lead and iron.

Potassium Permanganate: $^{N}/_{10}$ and $^{N}/_{100}$ standardised against pure sodium oxalate.

Glassware: All glassware was of pyrex and rinsed in many changes of distilled water which had been checked for the presence of iron.

Destruction of Organic Matter.

(a) Faeces. A 1 gramme sample of finely ground faeces was transferred directly to an 800 ml. Kjeldahl flask and completely washed into the flask by distilled water. 10 ml. of sulphuric acid and 2 glass-beads were added. The digestion, over a small micro-bunsen flame and in a fume-chamber, was allowed to proceed very slowly, the flask being rotated from time to time until a homogeneous mixture, free from foam, was obtained. At this point the speed of digestion was increased, adding frequently another 10-20 ml. of

sulphuric acid if necessary, until a golden-red, translucent solution resulted. 0.5 ml. of perchloric acid was then added and the digestion continued for a further hour when the solution was colourless. This process occupied 4-7 days.

(b) Whole Blood. 10 ml. of whole blood were used for analysis. The digestion was carried out in the sameway as for faeces; 10 ml. of sulphuric acid sufficed. To facilitate digestion the Kjeldahl flask containing the blood and sulphuric acid was allowed to stand overnight before digestion was commenced.

Reduction.

The contents of the Kjeldahl flask were diluted with 15 ml. water and raised to the boiling point with constant rotation. The sulphuric acid solution was then transferred quantitatively to a 125 ml. Erlenmeyer flask. 10 ml. distilled water were then added to the Kjeldahl flask, boiled and transferred quantitatively to the Erlenmeyer flask. The volume of transfer fluid should be kept as low as possible. Boiling out the Kjeldahl flask was absolutely necessary since, even with the greatest care, some etching of the glass occurred.

The contents of the Erlenmeyer flask were allowed to cool completely, and 5 grammes of granulated zinc were

added. The flask was then fitted with a Bunsen valve and allowed to stand overnight.

When the reaction between the zinc and sulphuric acid was complete, a fraction of a drop was removed with a capillary tube and tested for ferric iron with ammonium thiocyanate, using a porcelain spot plate. Very rarely was the addition of another 5 grammes of zinc necessary to complete reduction.

Titration.

When reduction was complete the contents of the Erlenmeyer flask were filtered quantitatively through a number 42 Whatman filter-paper. The flask was washed by decantation and the final residue transferred by a stream of water to the filter-paper. The filtrate including the washing was collected in a 400 ml. pyrex beaker which was embedded in ice to which freezing salt had been added. The washing and transferring were done as quickly as possible to avoid re-oxidation of the iron.

2 ml. of sulphuric acid were added to the contents of the beaker and titration with N/10 $KMnO_4$ carried out. The end-point was taken as the first recognisable pink flush persisting for 30 seconds. To facilitate identification of the end-point the beaker was painted white over half of the wall and over the bottom. When the amount of

iron was less than 10 milligrammes it was found advisable to use $^{N}/_{100}$ $KMnO_4$. All titrations were done with a Folin micro-sugar burette of 5 ml. capacity, graduated in 0.01 ml. to facilitate splitting of the drops.

Blank.

A blank was run on all reagents by carrying through the exact procedure without the addition of the iron-containing material. This blank was subtracted from the total titration figure.

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