## SECTION V

## DISCUSSION, SUMMARY & CONCLUSION

## CHAPTER 28

# D I S C U S S I O N

Comparable groups of patients as regards the age, sex and haemoglobin level were selected, anaemias of pregnancy being excluded because intolerance of oral iron is much more common in pregnancy than at any other time.[94] The selection of patients was strictly carried out as per the special criteria laid down for the purpose.

The patients were asked to swallow the iron tablets in my presence in order to confirm the ingestion of the full given dose and that too at a particular time so that 4 hours and 8 hours blood samples for serum iron estimation can then be punctually collected. They were

also given the opportunity of discussing the symptoms at each visit, although leading questions were reserved for the final visit. No patient having been treated once with ferrous sulphate or ferrous fumarate has been excluded from this series on the grounds of failure to respond or toxic effects.

A comparative study like this must ideally be carried out under standard conditions. Accordingly, various factors in the two series (Series A and Series B) were kept at identical levels, for example, age (adults), haemoglobin level (3 to 6 gm.), diet (identical in protein supply and elemental iron content), form of iron preparation (tablet form for accuracy of dosage), same disintegration time(non-coated tablets), composition of the tablets of ferrous sulphate and ferrous fumarate (only iron compound plus excipient), dosage (equal in terms of elemental iron),time of administration (immediately after meals), and the duration of treatment (3 weeks).

The urinary excretion of iron is increased by the administration of an acid salt and markedly decreased by the administration of an alkaline salt (Barer and Fowler, 1949). Moreover, administration of salicylates produces a fall in serum iron (Izak et al., 1962). It is because of these considerations that the patients were not given any mixture (including mist acid pepsin), and also that

the nursing staff was specially instructed not to give any medicament to any of the patients under present study. However, mist glucose was routinely administered to all the patients as a placibo and it may be emphasized that such administration could produce a definite and positive psychological feeling of improved digestion and/or sense of well being.

This subject including the methods for standardisation, serum iron estimation, estimation of elemental iron per tablet of iron preparation etc. is extremely complicated but all these methods have been simplified as far as possible and presented here in an easily understandable and practicable manner. A few points, however, might have been unavoidably repeated at different places because mention of such points at that particular place was necessary either for simplification or at least for the sake of completion. Nevertheless, sequence and simplicity have been possibly observed.

It is usual to obtain an optical density of 0.03 to 0.04 on analysing a sample containing 100 mcg. iron (Fe) per 100 ml. (Trinder, 1955). The optical density obtained here is 0.035 per 100 ml. and is, therefore, in close agreement with that found by other workers.

The method adopted for serum iron estimation (Marrack, 1956) is much modified during this study. All the details of the modifications have already been fully described in chapter 19. The method by virtue of all the modifications, has been rendered very easy. Moreover, it does not involve heating, extraction with organic solvants, daily preparation of reagent or multiple centrifugations. It is applicable to either serum or plasma.

A few of the photographs included in the thesis are of relative importance of maintaining the continuity of the photographic demonstration of various steps rather, than of absolute importance. This is particularly true so far as the photographic demonstration of various steps of detailed prodedure for serum iron estimation is concerned.

The signs and symptoms of iron deficiency are mostly due to the anaemia, which is microcytic and hypochromic, and is characterised by low M.C.H.C. The sore tongue, atrophic skin and nail changes found in iron deficiency anaemia are possibly due to a reduction in the amount of iron-containing enzymes which are necessary for the renewal of epithelial cells. Premature graying of hairs appears to be a frequent finding associated with iron deficiency anaemia.

Fever is unusual except in severely anaemic patients, in whom a low-grade elevation of temperature may be found. The spleen is palpable in about one-third cases of iron deficiency anaemia in later life. Excessive enlargement, however, is unusual. The spleen was palpable in only 2 patients of the present series.

Amenorrhea found in the patients of iron deficiency anaemia was not physiological but was due to the effect of anaemia. It appears to be the nature's effort to conserve whatever little amount of iron is present.

Besides the absolute figures, the percentage values of various findings have also been calculated and given at different places. Nevertheless, it may be emphasized that the series is too small to find and express the results in percentage.

## MECHANISM OF HYPOFERREMIA

The serum iron concentration is decreased in iron deficiency anaemias because there is in-sufficient metal for haemoglobin synthesis and the bone marrow is drawing on all available labile iron stores.(On the other hand, the serum iron value is increased in pernicious anaemia during relapse because little of the metal is being

utilised for haemoglobin synthesis). The serum iron level dinimishes in anaemia of infection because a metabolic defect results in transfer of absorbed iron to the storage depots.[23]

Salicylate administration results in immediate and constant fall in serum iron (Izak et al., 1962).In searching for the mechanism responsible for this decrease in serum iron, one has to consider first the possibility that the salicylate or its metabolites in the plasma might interfere with the determination of serum iron. This possibility was ruled out by the control experiments in vitro in which serum iron was estimated in a normal sample to which varying concentrations of aspirin or sodium salicylate was added.

Two other possibilities may be considered in this respect, viz. blood lost through venipunctures to obtain blood samples and/or blood lost through gestro-intestinal bleeding. Actual measurement revealed that the average blood loss by venipunctures was 6 ml. per day whilst that by gestro-intestinal was 3 ml. per day. These possibilities were ruled out by estimating the serum iron in volunteers who were bled upto 9 ml. on an average per day for 15 days, with no significant changes in serum iron during this period, and a prompt drop in serum iron after salicylate administration.

There was neither impairment of iron absorption as shown by the tracer studies, nor was there an increased excretion of iron in the urine during salicylate administration.

Thus, the fall in serum iron could not be explained by chemical interference with the determination, by blood loss, impaired absorption or increased excretion of iron. The cause of the fall in serum iron values in patients receiving salicylates, therefore, remains unclear.

Izak et al (1962) believed that the salicylates alter the function of reticulcendothelial system, as a result of which circulating plasma iron is removed from the circulation and deposited in the reticuloendothelial cells.

Bach et al (1952) pointed out certain resemblances in the biochemical effects produced by salicylates and cortisone. Sayers (1950) has suggested that salicylates exert their effects by promoting the utilisation of adreno-cortical hormones paripherally in the tissues and that this leads to increased production of ACTH. Moreover, injection of ACTH was followed by a significant fall in plasma iron in dogs (Cartwright et al., 1951) and in rabbits (Gupta et al., 1960). It appears reasonable, therefore, to conclude that adreno-cortical hyperactivity induced by salicylate administration is responsible for

the fall in serum iron.

Gupta et al (1959) observed that hypoferremia, in general, is associated with a febrile state. In patients with infection suffering from fever, there was marked hypoferremia. In experimental animals, the fall in plasma iron was maximum at the height of fever, 24 hours after the introduction of infection. In human volunteers each injection of typhoid vaccine was followed by a paroxysm of fever and profound fall in plasma iron. In rabbits in whom sterile inflamation was produced by turpentine injection, rise in body temperature was followed by hypoferremia. On the other hand, physical hyperthermia was unattended by any reduction in plasma iron.

This would mean that the fall in plasma iron is not due to a rise in body temperature per se, but may be due to other associated factors which lack in physical hyperthermia. That hypoferremia is not secondary to rise in body temperature is further suggested by the fact that patients of infection without any fever also showed a fall in plasma iron; and in rabbits injected with turpentine, when the temperature was at its height, plasma iron had returned to normal.

Bendstrup (1953) demonstrated that fall in serum iron in patients with chemically induced a fever was greater than in those with physically induced fever.

Accordingly, the hypoferremia occurred in various situations mentioned above may be related to the release of some chemical factor.. Inflammation, both bacterial or sterile, are characterised by tissue damage which is known to be associated with rise in body temperature (Roberts, 1954). For example, severe traumatic injury and cardiac infarction are associated with fever. It is significant in this respect that hypoferremia has been described to accompany both these conditions (Cartwright and Wintrobe, 1952; Feldthusen and Lassen, 1954).

The mechanism responsible for fever in infection is not understood. Recently, strong experimental evidence has been put forward that endogenous pyrogen derived from polymorphonuclear leucocytes in inflammatory exudates plays a central role in the pathogenesis of many kinds of fever (Wood, 1958). Presumably, some relationship might be existing between this pyrogen and hypoferremia seen in infections.

Another possibility is that both hyperthermia and hypoferremia may be the manifestation of systemic reaction to infection and as such, under the control of a common regulatory mechanism. It has been shown that ACTH can produce a fall in serum iron and that adrenals are actively concerned in the production of hypoferremia (Cartwright et al., 1951). Adreno-cortical hyperfunction

induced by injection of ACTH was followed by hypoferremia in rabbits (Gupta and Kumar, 1950).

Patients with infection and experimentally infected animals show hypoferremia and decrease in the concentration of iron-binding protein. It has been observed that hypoferremia develops as early as 24 hours after the experimental infection and in patients as early as 72 hours after the onset of illness (Kumar et al.,1958). Moreover, it is known that intravenous administration of iron in massive doses does not relieve the hypoferremia of infection (Kuhns et al., 1950).

It has been shown that in patients with infection the rise in plasma iron following a standard dose orally is very little as compared to normals (Brochner-Mortensen and Stein, 1942; Buchmann, 1944; Cartwright et al.,1948; Nilsson, 1948; Kumar et al., 1959). Failure of plasma iron to rise does not necessarily mean that its absorption is impaired because it can as well happen when the rate of removal of iron from the plasma is accelerated. Lately, however, it has been shown that in infections, the absorption of iron from the gastro-intestinal tract is considerably diminished (Gubler et al., 1950). If it be admitted that iron absorption is impaired in infections, even then it is unlikely that it is a major factor in the production of hypoferremia of infection.

It is known that the rate of removal of iron from plasma is twice as rapid in patients with infection as in normal subjects (Cartwright et al., 1948; Kumar et al., 1959). Thus, it appears that the main factor responsible for hypoferremia in infection appears to be the increased rate of iron removal from the plasma.

The next question that arises is the site to which iron is transferred from the plasma. There was no significant increase in urinary iron in patients with infection (Kumar et al., 1959). Thus, urinary excretion is rulled out. The work of Menkin (1930) and Menkin and Menkin(1931) drew attention to an increased accumulation of iron at the site of inflammation. But this possibility is also ruled out since it is not in agreement with many of the other workers (Greenberg et al., 1947; Kuhns et al, 1950; Kumar et al., 1959).

The iron content of liver, spleen and bone marrow is considerably increased in infected animals (Kumar et al., 1959). Perhaps, the same takes place in human infection. It appears reasonable to suppose that increased amounts of iron are deviated from the plasma to these tissues under the influence of infection. It has been suggested that the functional activity as also the demand for iron of the reticulo-endothelial cells is increased in infection (Vannotti and Delachaux, 1949; Cartwright et al., 1950), and that in infections, iron may have some part to play

in the defence of the organism (Vinnotti and Delachaux, 1949). It is, therefore, reasonable to speculate that since the reticulo-endothelial system has a vital role to play in the defence of the organism, increased amount of iron may be required by this tissue under the influence of infection.

In this context it has been shown that the blocking of Reticulo-endothelial system results in hyperferremia(Cartwright et al.,1950). Kumar et al (1959) studied the role of reticulo-endothelial system in the regulation of plasma iron and observed that the 'blockade' of R.E.System by injection of vital dye like Evans blue results in hyperferremia.

Since, it is generally recognised that vital dyes 'blockade' the R.E.system (Jungeblut and Berlot, 1926;Tuft, 1934; Gabrieli and Holmgren, 1952), it appears reasonable to conclude that hyperferremia resulting from injections of Evans blue was due th the accumulations of this dye in macrophages. The rise in plasma iron may then be explained by assuming that at least some portion of the non-haemoglobin iron of blood,whether derived from ingested food or normal haemoglobin breakdown,is deviated to the R.E.cells under normal physiological conditions.Loading of these cells with Evans blue resulted in failure to accept iron and hyperferremia followed as a sequence.Similar finding has been reported to follow the injection of colloidal thorium dioxide (Cartwright et al.,1950).

It has been shown that 'blockade' of R.E. system by vital dyes does depress its capacity to phagocytose the bacteria and particulate matter (Gay, 1930-31), abrogate the animal resistence to tumour implants and metastases (Foulds, 1932; Saphir and Appel, 1943; Ghosh, 1957) and impair its antibody-forming function (Issacs, 1925; Roberts, 1929; Tuft, 1934). The role of these cells in normal iron metabolism in the form of haemoglobin breakdown and formation of bile pigments and haemosiderin appears well established (Florey and Gowans, 1958). It is, therefore, reasonable to assume that hyperferremia observed to follow the 'blockade' of R.E. system may also be due to the depression in its functional activity.

It was further observed that previous injection of Evans blue abolished the hypoferremic effect of turpentine injection which acts by producing sterile inflammation. This would imply that the drop in plasma iron under the influence of turpentine is brought about by an increase in the transfer of iron from plasma to R.E. cells. Possibly, a similar mechanism operates in infections. An increase in the iron content of tissues rich in R.E. cells e.g. Spleen liver and bone-marrow of animals subjected to experimental infection has already been referred to.

It is true that R.E. cells utilise iron for their function, and their need for iron would grow in conditions

associated with increased R.E.Cell activity. It has been suggested that R.E. system is of vital importance in the promotion and maintenance of body well-being in pathologic states (Bogomoletz, 1943). Since R.E. cells are concerned in the defence mechanism of an organism and since iron is an important constituent of cellular enzymes, these cells are likely to require more iron for an increase in their function consequent to infection. As such, large amount of iron may be deviated to these cells resulting in low plasma iron levels in infectious diseases. The failure to induce hypoferremia by experimental inflammation in rabbits with 'blocked' R.E. system (vide supra) lends support to this hypothesis.

It has been shown that hypoferremia is not a specific systemic reaction of an individual to infection but widely divergent stimuli such as, electric shock and operative trauma also produce hypoferremia (Gupta et al., 1960). Hypoferremia has also been reported to occur after injections of turpentine and typhoid vaccine (Gupta et al., 1960), and in patients with coronary occlusion and traumatic injuries (Feldthusen and Lassen, 1954). Adreno-cortical hyperfunction induced by injection of ACTH was followed by a significant fall in plasma iron in dogs (Cartwright et al.,1951) and in rabbits (Gupta et al., 1960). Furthermore, accompaniment of eosinopoenia with hypoferremia in patients subjected to operative stress is suggestive of an associated adrenocortical hyperactivity (Gupta et al., 1960).

From these observations, it is clear that certain conditions, although clinically quite unrelated, show hypoferremia as a common biochemical change. Whether there is a single or multiple factors responsible for this alteration common to a variety of divergent stimuli is not known. All the conditions mentioned above produce a state of distress in the organism. It appears likely that "stress" caused by these stimuli is in some way concerned in the production of hypoferremia. It is note-worthy in this context that hypoferremia has been reported to occur in association with general adaptation syndrome (Selye, 1950). Whether this change is brought about by an increased activity of the reticulo-endothelial system, or adrenal cortex, or through an entirely different mechanism cannot be definitely stated.

Whether hypoferremia in infections and other conditions is influenced by adreno-cortical hyperactivity cannot be inferred from these observations. Nevertheless, the accompaniment of ecsinopoenia with hypoferremia in patients subjected to operative stress lends some support to the view that there is possibly an increase in the adreno-cortical activity.

## HOOKWORM ANAEMIA

The blood picture and clinical state in hookworm anaemia possess all the characteristics of iron deficiency

anaemia and the anaemia can be relieved by the administration of iron.[139] The findings and results of the present study are in agreement with this in toto.

Erulkar et al opined that, in some cases of ankylostomiasis, the anaemia was megaloblastic and resembled pernicious anaemia. It may be emphasised that this opinion was not based upon the marrow biopsy, but on high values (more than 1) for colour index and the presence of 'megaloblast' in the peripheral blood smear. Daftary and Bhende (1956) suggested that ankylostomiasis, per se, does not produce a megaloblastic anaemia, but megaloblastic marrow may be found in complicated cases i.e. in cases of megaloblastic anaemia complicated by superadded hookworm infection.

It is generally believed that specific anthelmintic treatment should not be started if the haemoglobin is below 30%. In such a case, anaemia should be treated first with iron and after the haemoglobin rises above 50 per cent, specific anthelmintic should be given.

In our past experience, however, the use of tetrachlorethylene for treating the uncomplicated cases of ankylostomiasis associated with even severe anaemia did not give rise to any significant toxicity. Moreover, patients with haemoglobin as low as 1.5 gm. per 100 ml. of blood, massive edema and cardiac failure have been given the full dose of the drug without untoward happenings.[33] Parekh and

Rane (1958) observed that some cases of hookworm infection do not give haematological response to iron therapy unless prior deworming is carried out.[95] This might presumably be due to the amount of blood loss and iron loss associated with hookworm infection (Chapter 8). Lane (1937) suggested that continued infestation does not allow the haemoglobin figure to be maintained at a normal level.[133]

Thus, "prior deworming" is not only safe and advantageous but also is a 'must' to prevent iron loss which is a very important consideration in a comparative clinical therapeutic trial of two different iron preparations like this, because then and then only, it is possible to give full justice to the comparison of the haematological responses obtained after the administration of respective iron compounds.

It was, therefore, decided to carry out deworming as well as to confirm complete irradication of parasites BEFORE starting the iron therapy i.e. before commencing the actual comparative clinical trial of Ferrous Sulphate and Ferrous Fumarate.

Tetrachlorethylene ($Cl_2$:C = C : $Cl_2$) is now the drug of choice for the treatment of hookworm as it is safe, effective and cheap. It is not absorbed to any appreciable extent except in the presence of fats or alcohol.

It is non-toxic in anthelmintic doses.

Millions of patients, many of them anaemic and cachectic, have been treated with this drug. The several reports of untoward effects of tetrachlorethylene relate to nausea, drowsiness, giddiness and headache. The only reported death was in an extremely debilitated and emaciated beggar in India. Carr and his associates reported the administration of 591,000 hookworm treatments with tetrachlorethylene without untoward effects other than occasional giddiness and drowsiness.[33] No untoward effects have been observed in the present series too, inspite of the fact that the drug was administered to patients with low haemoglobin level (3-6 gm. per cent).

If an Ascaris infection is present with the hookworms, Ascaris should be removed by piperazine before the hookworm treatment is given, because tetrachlorethylene may stimulate Ascaris to migrate or produce intestinal obstruction.[42] This point is of great practical importance.

## AETIOLOGY OF IRON DEFICIENCY ANAEMIA

As regards the aetiology of iron deficiency anaemia, it can be seen that the incidence of ankylostomiasis in the present series is cent per cent, besides the associated factors like deficiencies of diet and multiple pregnancies.

Rightly says Manson-Bahr,[86] "I am one of those who are in mild disagreement with some modern high-powered haematologists who would ascribe most forms of anaemia in the tropics to dietetic causes, thereby trying to debunk the role of ankylostome (and the subtertian malarial parasites) as the main causes."

There is no doubt that there is a racial factor. The Fijians, who are heavily infected with hookworms (27.8 per cent) seldom if ever, suffer from anaemia, and this has been verified by Wills and Bell. On the other hand, the Indians who are domiciled in Fiji frequently suffer from anaemia.

It has been suggested that each worm is capable of drawing about 0.67 ml. of blood and in heavy infections such daily withdrawal of blood by a large number of parasites over a prolonged period would be sufficient to cause anaemia. Darling estimated that 1 per cent haemoglobin can be lost per one dozen of worms.[35]

Roche et al (1957) estimated that the average loss of blood by the host, per hookworm per day is 0.2 ml.[35] It is true that Ankylostoma consumes more blood than Necator.[33] Layrisse et al. (1961) using $Cr^{51}$ and $Fe^{59}$ marked red cells found that the blood loss per day in the stools varied from 2 to 250 ml. in rough proportion to the severity of infestation.[133] Roche et al (1959)estimated

the intestinal iron loss and reabsorption and found that considerable amount of iron was reabsorbed from the blood lost in intestinal tract, but the net loss was substantial nevertheless.[112]

At one time the view was widely held that hookworm anaemia is due to the elaboration of a toxin. No such mysterious concept is necessary because there is no evidence that the parasite produces a haemolysin or that intravascular haemolysis plays any part in the production of anaemia.[133]

As regards malaria, the present anti-larval and hygienic measures have been so widely applied, aided by the much improved methods of drugs prophylaxis and treatment, that a state of affairs has been created where the malarial anaemia, which was so common previously, exists no more.

The role of the skin as an additional and important channel for iron loss in relation to iron deficiency anaemia has not received as much attention as the problem deserves. Hussain et al. (1960) suggested that this could be one of the etiological factors in the widespread prevalence of iron deficiency anaemias in the tropics.

The dermal loss of iron can occur through insensible perspiration as well as through active sweating. A proper appreciation of the excretary role of the skin is

important in balance studies in which intake and output of iron are determined particularly in tropical climates in which sweating occurs normally.

Active sweating is accompanied by desquamation of the superficial layers of epidermal cells and hence sweat always contains epithelial debris. Thus, the major portion of the loss of iron is primarily associated with desquamation probably of cell lining the sweat glands.

Different investigators have found different figures for iron excretion in sweat, the discrepancy between the results being due to the different techniques employed for iron estimation. Mitchell and Hamilton (1949) and Adams, Leslie and Levin (1950), found that the loss of iron in 'cell rich' sweat was high amounting to over 6 mg. per day although according to latter authors 'cell free' sweat contained negligible amounts of iron.

Johnson, Mc Millan and Evans (1950) found the iron losses amounting to 0.04 to 0.12 mg. per hour in conditions of profuse sweating. Foy and Kandi (1957) found the iron losses amounting to 0.3 to 6.0 mg. iron per litre in 'cell rich' sweat and 0.1 to 0.2 mg. iron per litre in 'cell free' sweat.

Hussain et al (1960) found the iron losses amounting to 0.63 to 1.88 mg. with a mean of 1.15 mg. iron per litre of 'cell rich' sweat and 0.15 to 0.53

mg. with a mean of 0.34 mg. iron per litre of 'cell free' sweat. Even assuming that these values were representative for Indians, as much as 15 mg. of iron can be lost per day under conditions of maximal sweating.[64] Although the amount of iron in 'cell free' sweat is considerably less than that in 'cell rich' sweat, it also might assume great significance in the tropical hot humid climate where people may sweat profusely to the extent of 2 to 11 litres per day (Kuno, 1934).

India lies partly in the tropics and partly in the subtropics with extreme variations in climates. In the plains of India sweating is a common occurrence during summer and more so in times of high humidity. There are regions where hot and humid climate prevails throughout the best part of the year. It is in these regions that losses of iron through sweat are expected to be appreciable.

Thus, it is clear that the dermal loss of iron can be one of the possible contributory factors in the genesis of iron deficiency anaemia in the tropics.

Another important etiological factor for iron deficiency anaemia seems to be the consumption of salicylate compounds. Cases of "Salicylate Anaemia" have been studied and recorded by Summerskill and Alvarez (1958). Habitually heavy consumption of salicylates for headaches had xxxx

coincided with the onset and the subsequent course of the anaemia in such patients. Occult bleeding from gastro-intestinal tract was demonstrated during controlled periods of salicylate medication. After salicylate consumption had been greatly reduced, anaemia did not recur during the follow up periods.

The possible causes of bleeding after salicylates include erosive or haemorrhagic gastritis (Douthwhite and Lintott, 1938; Muir and Cossar, 1955), but acute lesions can also occur lower in the gastro-intestinal tract. Patients with chronic peptic ulcer or previous dyspepsia are particularly prone to massive haemorrhage (Muir and Cossar, 1955; Alvarez and Summerskill, 1958), perhaps because more severe lesions, result from increased acid-pepsin production by the stomach or impaired mucosal resistance, but occult bleeding apparently occurs equally in all groups of patients (Alvarez and Summerskill, 1958). Moreover, the use of soluble aspirin fails to eliminate the risk of massive or occult gastro-intestinal bleeding (Alvarez and Summerskill, 1958).

The incidence of anaemia due to occult bleeding can occur from salicylates is unpredictably, but both salicylate consumption and iron deficiency anaemia are common. Two factors are probably important, and both might contribute to the development of anaemia. The risk is likely to be related to the amount of salicylates

consumed, and it would therefore be greatest in patients with long-standing painful disabilities such as, headache or musculoskeletal disorders. In addition, anaemia may develop more readily under these circumstances in patients with pre-existing impairment of iron absorption or utilisation.

Izak et al (1962) suggested the possibility that salicylates, in addition to their effect on the mucous membranes of gastro-intestinal tract, might otherwise interfere with the metabolism of iron. They, therefore, studied the iron metabolism by estimating serum iron during salicylate administration and found that the most immediate and constant result of the salicylate administration (3-4 gm. of Aspirin per day) was a drop in serum iron. However, a considerable variation appeared to exist both in the time of onset and the magnitude of this drop. The decrease in the average values before and during administration ranged from 11 - 61 per cent. The maximum drop ranged from 35 to 78 per cent of the original serum iron concentration. The drop started to occur on 2nd to 7th day and became maximal on the 9th to 36th day after the administration of aspirin was instituted. After the salicylate administration was discontinued, the serum iron rose again in almost all the cases. Besides the fall in serum iron, most of the patients in whom these studies

were carried out showed a decrease in haemoglobin, haematocrit and red cell survival time. These changes[66] are shown in the following table:

TABLE NO. 47

AVERAGE VALUES FOR SERUM IRON, HAEMOGLOBIN AND HAEMATOCRIT BEFORE, DURING AND AFTER SALICYLATE ADMINISTRATION

| Case No. | Sex | Serum iron r% | Haemoglobin gm% | Haematocrit % |
|---|---|---|---|---|
| 1 | Male | 110-62-85 | 15.1-14.9-14.8 | 47-46-45 |
| 2 | Male | 123-82 | 14.8-15.2 | 47-48 |
| 3 | Male | 126-82-109 | 14.1-13.8-14.2 | 47-43-47 |
| 4 | Male | 89-79-84 | 15.2-14.7-14.7 | 48-46-44 |
| 5 | Female | 153-72-79 | 13.6-12.7-12.4 | 44-41-41 |
| 6 | Male | 90-60-66 | 14.7-14.3-14.0 | 47-45-42 |
| 7 | Male | 138-98-88 | 14.0-13.1-12.5 | 43-42-41 |
| 8 | Female | 140-55-86 | 15-12.2-11.9 | 46-40-41 |
| 9 | Female | 119-59 | 14.2-13.2 | 44-42 |
| 10 | Male | 71-38 | 12.4-12.0 | 41-40 |
| 11 | Female | 63-45-46 | 12.2-11.2-11.0 | 42-38-37 |

The diagnosis in these cases was as follows:

| Case No. | Diagnosis |
|---|---|
| 1,2 | Normal |
| 3 | Diverticulosis Colon |
| 4,5,6 | Essential hypertension |
| 7 | Atherosclerosis |
| 8 | Rheumatic heart disease |
| 9 | Neurofibroma of Cauda equina |
| 10 | Rheumatoid arthritis |
| 11 | Osteoarthrosis of spine |

Since the amount of blood lost through the gastro-intestinal tract (about 3 ml. per day) was not sufficient to explain the hypoferremia and anaemia observed, the possibility must be considered that the bleeding may have contributed to the decrease in circulating haemoglobin in these patients during salicylate administration. The combination of blood loss and increased red cell destruction may account for the anaemia which developed following salicylate administration.

These observations form an additional indication that the prescription of even a common and apparently innocuous compound may have more profound effects on the body economy than is generally realised. Moreover, the multiplicity of salicylate compounds readily available to the public under different names, and the difficulty of finding equally effective non-salicylate analgesics, often results in self-medication with salicylates. These considerations stress the importance of eliciting a detailed history regarding prescribed therapy and self-medication, particularly in patients with iron deficiency anaemia and in subjects to be selected for serum iron estimation studies, as has been done in the present study.

Among the several factors which interfere with the digestion and absorption of food iron, the phytic acid and phytate content of the diet are important. The normal Indian diet is based upon cereals and pulses which form

the principal sources of phytate. Foy and Kondi (1957) stated that the normal Indian diets contain approximately 1000 mg. P in the form of inorganic phosphate and 1000 mg. as phytin phosphate and concluded that with all probability the iron would be precipitated either as insoluble phytate or phosphate.

Hussain and Patwardhan (1959) found that when the dietary iron intake was kept constant at 22 mg. per day, the average iron absorption at 8 per cent level of phytate was much greater than that at 40 per cent level of phytate. This has been shown in the following table :

TABLE NO. 48

IRON ABSORPTION ON LOW AND HIGH PHYTATE DIETS

| Dietary Phytate per cent | Iron Intake mg. | Iron in Faeces mg. | Iron absorption mg. |
|---|---|---|---|
| 8 | 22 | 18.62 | + 2.48 |
| 40 | 22 | 21.10 | + 0.173 |

Apte and Venkatachalam (1962) kept the usual phytate content of the cereal diet constant at 40 per cent level, and observed that an intake of 11.7 mg. of iron per day is insufficient to meet the daily requirements; that an intake of 16.43 mg. of iron per day is just sufficient

because the subjects remain in a delicate balance with regard to their iron need; and that an intake of 21.55 mg. of iron is sufficient because about 30 per cent of the intake is absorbed producing a distinct positive iron balance. This has been shown in the following table :

TABLE NO. 49

IRON ABSORPTION AT THREE DIFFERENT LEVELS OF IRON INTAKE

| Dietary phytate per cent | Iron Intake mg. | Iron in Faeces mg. | Iron Absorption mg. |
|---|---|---|---|
| 40 | 11.72 | 12.52 | - 0.80 |
| 40 | 16.43 | 16.04 | + 0.39 |
| 40 | 21.55 | 14.55 | + 7.10 |

Thus, inspite of normal diet and dietary intake of iron, the poor absorption of iron from the intestine due to high phytate content, and especially so in the vegetarian diet, can play an important role in the genesis of iron deficiency anaemia.

The importance of the hydrochloric acid of the gastric juice has been the subject of debate since long and there are many conflicting observations:

(a) An acidity has little or no effect on the absorption of iron in therapeutic doses (Fowler and Barer, 1937), and the addition of HCl in therapeutic doses does not affect absorption.[94]

(b) Achlorhydria does not usually influence the response to therapeutic doses of iron. Nevertheless, it may well have a significant and adverse effect on absorption of dietary iron.[26]

(c) Moore and Dubach (1951) have shown that the amount of radio-active iron absorbed was not influenced by the presence or absence of acid. Other workers have reported findings contrary to this.[105]

These and many other conflicting observations can be resolved if one assumes that the facilitation of iron absorption attributed to the normal acid secretion of stomach is real but, nevertheless, relatively unimportant under normal circumstances. However, when the requirement of iron becomes relatively high, the presence of free hydrochloric acid may be important in making additional dietary iron available for absorption. Thus, hydrochloric acid is only of minor importance except perhaps where iron requirements are high and therefore even minor factors may be significant. Although it is true that many normal individuals can be found who have achlorhydria

and yet show no anaemia and inspite of the fact that iron deficiency is uncommon in pernicious anaemia where achlorhydria is characteristic, it is noteworthy that chronic hypochromic anaemia is not unusual in men following gastrectomy even though this type of anaemia is rare in the male sex otherwise. Hypochromic microcytic anaemia is more common following this operation than macrocytic anaemia both in man and in experimental animals. Such animals were found to be particularly prone to develop anaemia during pregnancy.

It appears that achlorhydria alone will rarely cause iron deficiency but, when other factors are contributing to the development of iron deficiency and, in consequence, iron requirements are increased, the handicap of achlorhydria may be of some importance.

The effect of a single haemorrhage, even if severe, is transitary in a normal individual with adequate iron stores. Compensatory blood dilution occurs, the marrow is stimulated to a higher level of erythropoiesis, and the iron stores are mobilised. In a few days, enhanced erythropoiesis and haemoglobin formation reach a peak and many young cells appear in the blood. In a few weeks the haemoglobin level is again normal and erythropoietic activity declines. Recovery is complete, although the slow replacement of iron stores may require months and years.

Repeated blood losses soon lead to exhaustion of the iron stores. In the absence of treatment, a precarious balance may then be established. Intestinal iron absorption is increased, iron transport is accelerated and total haemoglobin formation may rise. Nevertheless, the amount of haemoglobin formed is insufficient to meet the increased demand. As the condition progresses, the marrow eventually becomes hypoplastic. Without treatment, such patients can go on for a long time with an extreme degree of anaemia. Gradually, iron-protein conjugates (iron enzymes and myoglobin) are affected. Undoubtedly, all cells suffer and particularly those which divide frequently.

In men, iron deficiency anaemia has been regarded by many authorities as invariably the result of pathological blood loss. The practical worth of this point is undisputed and a search should always be made for the lesion. Nevertheless, experience indicates that in males, even 'Idiopathic' hypochromic anaemia not due to blood loss does occur. This view has been supported by the findings of Bhatt (1959). The cause may be difficult to identify. A considerable proportion of cases show achlorhydria, inadequate intake or defective absorption of iron (even when the patient is taking a normal diet), chronic infection, excessive dermal loss of iron or salicylate administration, singly or in multiplicity, may be responsible.

## INVESTIGATIONS

Greater justification for iron therapy is provided by clinical evidence of iron deficiency and more extensive laboratory investigation, Koilonychia is by far the most reliable clinical indication of iron deficiency. It is not a feature of other types of anaemia such as pernicious anaemia. A combination of dysphagia and glossitis is a probable indication of iron lack but is not common enough to be useful as a general guide. Glossitis and marginal stomatitis may occur in association with hypochromic anaemia but are not reliable evidences of the cause of anaemia as they often occur in anaemias such as, pernicious anaemia when there is no iron deficiency whatsoever.

The simpliest laboratory evidence on which iron deficiency anaemia can be postulated is the estimation of haemoglobin and the examination of the blood smear. Red cell counts are liable to considerable error and particularly so in the hands of those without experience, whereas the smear has the advantage of allowing scrutiny of the red cells (size, shape and filling of haemoglobin), and the morphology of the white cells.

It is now generally accepted that because of the gross inherent errors, erythrocyte counts do not serve a useful purpose. Statistical analyses indicate that the

probable minimum error of red cell counts is ± 7.8 per cent, even in the hands of trained technicians.[137] According to Todd et al., this is true for only 66 per cent observations.[125]

The colour index is only a crude indication as this index depends upon the cell count and haemoglobin content. This limitation in the value of colour index is not yet well recognised in general practice and the index may be entirely misleading.[68]

These considerations lead to question the usefulness of indices like M.C.V. and M.C.H. which are based upon the erythrocyte count. Since the red cell count is so often in error, gross inaccuracies may occur in the determination of M.C.V. Briggs and Macmillan[24] concluded that the probable minimum error is 7 to 9 per cent. One can go even further and say that in some hands the calculation of M.C.V. is worthless, and can be misleading.[137]

As compared to M.C.V. and M.C.H., M.C.H.C. is much more reliable as it does not depend upon the erythrocyte count but is calculated from the haemoglobin and P.C.V., both of which have low statistical errors. The absolute value of M.C.H.C. is very valuable in deciding the deficit of iron, and this is a helpful guide for iron therapy.[68] It may be said that, inspite of all these drawbacks, the

indices are definitely useful as a guide for the further investigations and should, therefore, be calculated in all cases of anaemia.

Verghese et al. (1959) observed that there is a close correlation between the haemoglobin level and and corresponding M.C.H.C. in an uncomplicated case of iron deficiency anaemia. The results of the present study are also in agreement with this observation. To a certain extent, the rise in M.C.H.C. is directly proportional to the increase in haemoglobin on iron therapy. A disproportionate rise of M.C.H.C. to normal in relation to the haemoglobin value is an indication to search for any complicating factor. There are, however, certain fallacies. If the initial haemoglobin is below 4 gm., the error of the calculated M.C.H.C. is high, and hence the need for repetation of blood examination. Also, there is a change in the M.C.H.C. when a severe case of anaemia is under bed rest, possibly due to the changes in the blood volume. So it is advisable to repeat the blood examination three to four days after the admission and before commencing the iron therapy.

Undoubtedly, in all instances, whether the technique is beyond critism or where it is only mediocre, the examination of the blood smear by the physician himself is extremely important.[137] In this way gross errors are not

likely to pass unnoticed and a visual picture of the blood morphology can be obtained. In addition, sometimes important information may have been overlooked by persons less directly interested in the patient.

An increase in the percentage of reticulocytes is the earliest noteworthy effect on the blood. Their enumeration affords a simple and valuable method of determining the effectiveness of therapy. The total number of reticulocytes which are released from the bone marrow following the treatment appears to be related directly to the degree of bone marrow involvement. The speed with which they are released depends on the amount of effective anti-anaemic substance administered, and the rapidity with which it reaches the marrow. Thus, when an average therapeutic dose is given daily, the reticulocytes will usually begin to increase in number about 3 to 5 days after the commencement of treatment and a maximum percentage will be reached on the 8th to 12th day.[138]

If a massive dose is administered, the reticulocyte response may commence in 48 hours and reach a maximum in 104 to 140 hours. The height of the reticulocyte curve depends on the degree of anaemia, varying inversely with the number of red corpuscles and the speed of the response.[138]

There are two methods of comparing the reticulocyte response obtained after the administration of two different

iron preparations :

(a) To find out the actual rise of reticulocytes (i.e. Peak - initial) and then to compare the two figures as is done in this study.

(b) Direct comparison of peak (irrespective of the initial reticulocyte count). This comparison may not be accurate since it depends upon the initial reticulocyte count.

Leucocyte count was carried out in each case, primarily to rule out the presence of infection by excluding leucocytosis, and to find out the presence and extent of cosinophilia in cases of ankylostomiasis. It was also done in each case to see whether a reduction in R.B.C. count affected the W.B.C. count. The results showed that every case of severe anaemia is not necessarily associated with leucopenia.

The platelet count has been found to be at the lower limit of the normal range in most of the patients of iron deficiency anaemia, or even slightly reduced in some of the patients.

The range of serum proteins obtained in the present series is 3.3 to 5.5 gm. per cent as against the normal range of 6 to 8 gm. per cent. Thus, the serum protein

estimations revealed the presence of varying degree of hypoproteinemia in all these cases. In accordance with this, a history of poor diet was also present in all the cases.

In recent years, the view that concentration of protein in the blood is a satisfactory evidence of the nutritional status of an individual is challenged by Pollack, Halpern and Keys. Equally significant is the experimental data of Whipple, that albumin is formed mainly from aminoacids of animal origin whereas globulin is formed mainly from amino-acids of vegetable origin.[16] These findings, along with the consideration that, even the non-vegetarian hospital class of patients, as observed in this series, are in reality taking vegetable products in greater amounts than the non-vegetarian products, probably explains Woodruff's (1950) observation that in cases of anaemia associated with Hypoprcteinemia, the albumin fraction is reduced and globulin fraction is increased as compared with the normal values.[140]

Though there is sufficient evidence to show that the concentration of proteins in the blood cannot be taken as a satisfactory index of nutritional status, the fact that the protein pattern of the blood is affected by the nature of the diet, gives significance evidence to show that the

nutritional status influences the blood protein pattern, and indirectlythe total protein concentration. Analysis of the results obtained by various workers also lends proof to this latter fact (Best and Vaidya, 1959).

In an attempt to establish the corelation between edema, anaemia and hypoproteinemia, it is found that edema is neither related directly to the severity of anaemia nor it is related directly to the severity of hypoproteinemia. All cases of anaemia with associated hypoproteinemia do not necessarily show edema.

It is well-known that the edema often complicates malnutrition, but its pathogenesis is not clearly understood. Starling's classical concept whereby physico-chemical alterations resulting from the changes in the plasma proteins were held responsible for the production of edema is now recognised as inadequate. Gopalan(1950) observed increased excretion of an antidiuretic substance in the urine of such patients with nutritional edema and oliguria. The response of such patients to a water-load was impaired, and so was that of laboratory animals on low protein diets (Gopalan and Ramnathan, 1957; Dicker et al., 1946). Normal liver was shown to be capable of inactivating the posterior-pituitary anti-diuretic hormone (Eversole et al., 1949), and this capacity was found to be impaired by low-protein diets (Birnie, 1950; Gopalan et al.,

1953). Profound structural and functional changes are known to take place in the liver as a result of malnutrition and edema is often an associated factor. On the basis of these observations a tentative hypothesis to explain the pathogenesis of edema was put forward by Gopalan and Venkatachalam (1952) and Gopalan (1955).

Baez et al. (1950) showed that the iron-protein complex ferritin can, under certain circumstances, stimulate posterior pituitary and thereby exert a considerable antidiuretic effect. Ferritin is stored mainly in the liver and spleen. These organs, under certain conditions, release measurable amounts of ferritin into the circulation. It is interesting to note in this context that the presence of ferritin in the blood has been reported in some clinical conditions, such as, congestive cardiac failure, toxaemia of pregnancy, cirrhosis of liver, nephrotic syndrome (Shorr et al., 1950), and in nutritional edema (Srikantia, 1958), which are all characterised by periods of oliguria and altered water metabolism. In the last-mentioned condition, the ferritin disappears from the blood after nutritional therapy.[120]

The presence of ferritin in blood at a stage when oliguria and edema are conspicuous in patients with edema associated with hypoproteinemia, and its disappearance after nutritional therapy, appear significant. Thus, it appears

that there exists a causal relationship between ferritinaemia and antidiuresis. It is, therefore, reasonable to postulate that hypoproteinemia releases ferritin from the damaged liver into the circulation which in turn stimulates posterior pituitary to release anti-diuretic hormone, thus resulting in fluid retention and subsequent edema.

As regards the urine examination, no abnormality was detected inspite of the fact that a special care was exercised not to miss the microscopic haematuria.

Stool examination of all the patients revealed the presence of hookworm ova, thus presenting a high incidence, as almost all the patients came from the poor class of population. Most of these patients were labourers in farm with a definite history of barefootedness.

In 40 cases of this series under study, all had hookworm infestation. Out of these 40 cases, 12 cases(30 per cent) gave the occult blood test positive and 6 cases (15 per cent) showed the presence of Charcot-Leyden crystals. Verghese et al. (1960) studied 32 cases, out of which 23 cased(about 70 per cent) had hookworm infestation and out of these 23 cases, 12 cases (about 50 per cent) gave the occult blood test positive.[128] In order to prevent false positive Benzidine test all the precautionary measures

were taken (Page 198). That the test was not false positive was further confirmed by the presence of plenty of R.B.Cs.in all the cases which gave positive benzidine test.

As described earlier, the stool in each case was examined by simple method and if necessary by concentration method (Brine Flotation Technique). This technique was first introduced by Bass in 1906 and was modified by Kofoid-Barber Brine in 1918.[36]

In most of the cases, direct wet preparation could reveal the presence of hookworm ova but whenever it used to fail to do so, Brine flotation technique gave the positive results. Hence the direct wet preparation along with the Brime flotatiCn technique may be used for obtaining best results for the detection of the helminthic ova. In this connection, it may be added that these results compare well with those of Shrivastav (1954), Vaishnav and Bhande (1955), and Acharya, Amin and Sayed (1959). Acharya et al (loc.cit.) have concluded that the Brine flotation technique is the best method for concentrating the helminthic ova.[1]

The microscopic examination of stool for hookworm is a time-consuming affair for the rapid surveys. This can be easily overcome by the specific intradermal test which consists of intradermal injection of specific

antigen. Thedefect of the microscopic examination of faeces for the diagnosis was realised by Hall (1911),who stated that it consumed time, caused strain on the eyes and needed much mental concentration.[101] No one with experience in this type of work will deny that the examination of a number of negative slides causes more strain to the mind and eyes than the examination of a similar number of positive ones.

The intradermal test, besides being quick and easy, has another advantage that field hookworm surveys in rural areas in India, for which special organisation for collection and miscropic examination of stools is needed, can be carried out by a trained technician in the village itself. The assessment of the prevalence of hookworm infection in the rural population is a good index of soil pollution and should be frequently used to evaluate the environment sanitation programme for the proper disposal of human excreta which is an important measure for the prevention of ankylostomiasis, which is so widely prevalent.

When reliable laboratory support is available, there is little difficulty in making a diagnosis of iron deficiency anaemia. A microcytic hypochromic picture seen in the stained smear is supported by a colour index below normal, a mean corpuscular volume below 80 cu and a mean corpuscular haemoglobin concentration under 30 per cent.

True iron deficiency anaemia is confirmed by estimating the serum iron and iron-binding capacity of the serum and also by carrying out the oral iron absorption test. Besides this, iron therapy is also a diagnostic and therapeutic test.

In the great majority of cases, however, haemoglobin deficiency is a manifestation of true iron deficiency and it is customary and justifiable to prescribe iron.

## IRON THERAPY

Until recent years, the only practicable route for iron administration was by mouth, and it is still the route of choice in the great majority of cases. The most commonly used iron preparations are ferrous sulphate and ferric ammonium citrate, and the respective daily doses commonly employed are 9 grains (0.55 gm.) and 90 grains (6.0 gm.). The choice of preparation is a matter of convenience and tolerance. Ferrous sulphate is more convenient, does not stain the teeth, and is cheaper than the ferric salts. Therapeutic doses of either of these preparations may induce epigastric pain, vomiting and diarrhea.

The practicability of intravenous injections of saccharated iron oxide was first demonstrated in 1949.

The initial popularity achieved by this form of treatment might be regarded as indicating its value and safety, but subsequent experience has shown that it was somewhat over-rated. The efficiency of the treatment is undisputed, but indications for its use are rare. Furthermore, intravenous iron preperations are expensive, troublesome to administer, and may cause pain at the site of injection and generalised toxic symptoms, such as, pyrexia, palpitation, vomiting, tachycardia, severe lumber pain and even a state of collapse.

There is every possibility that the treatment by the intramuscular route will supersede the intravenous administration. The introduction of an iron-dextran compound for intramuscular injection has removed many of the objections to the parenteral therapy. Of course, the need for such treatment is unaltered, but the low incidence of unfavourable reactions has allowed its administration as a matter of convenience when the intravenous preparation would not be used. As a rule, the intramuscular injection causes only slight discomfort. Local staining of the skin may result, especially if the injection is given too superficial. The staining may take several days to appear and it may do so at some distance from the injection site, its passage being along the fascial planes.

There is no doubt that the iron given by the parenteral route is efficiently utilised for haemoglobin synthesis.

In uncomplicated cases of iron deficiency anaemia, the rise in haemoglobin which follows treatment often exceeds 1 per cent per day. It is also established that very little of the administered iron is excreted, even if there is no iron deficiency in the body. Protracted parenteral administration of iron in excess of requirements will, therefore, lead to siderosis as a storage depots become saturated with iron and deposit haemosiderin. Although it is not yet clear what risk there is of fibrosis and parenchymal damage as a result of these iron deposits, it is obviously desirable to avoid any possible chance of inducing a state resembling haemochromatosis. Whenever iron is to be administered parenterally, an attempt should, therefore, be made to assess the needs of the case.

On the basis of calculation for the amount of iron required, adequate iron can be administered, and at the same time excessive treatment can be avoided.

The average effective dose of iron has been defined as a dose which produces an average increase of at least 1 per cent of haemoglobin per day.[41] To produce a rise of 1 per cent of haemoglobin, about 25 mg. of elemental iron should be absorbed. Knowing the haemoglobin deficit in a given patient, the amount of iron required to make up the particular deficit can be calculated.

The dosage can also be calculated from the M.C.H.C. deficit. The total dose of iron required to raise M.C.H.C by 1 per cent in cases of iron deficiency anaemia was found to be 172 mg.[129]

It has been shown that ferrous gluconate, ferrous succinate and ferrous fumarate are all efficient preparations, but not significantly more efficient than ferrous sulphate (O'Sullivan et al. 1955; Swan and Jowett, 1959). They are 5 to 7 times more expensive but they are on the whole better tolerated than ferrous sulphate although much of this is probably due to psychological factors.[132]

The cost of one course of any of these iron preparations, taking ferrous sulphate as standard, is as follows:

TABLE NO. 50

COMPARATIVE COST OF DIFFERENT IRON COMPOUNDS[119,123]

| Sr.No. | Iron compound | Cost |
|---|---|---|
| 1 | Ferrous Sulphate | 1.0 |
| 2 | Ferrous Gluconate | 2.2 |
| 3 | Ferrous Chelate | 2.9 |
| 4 | Ferrous Succinate | 3.9 |
| 5 | Ferrous Fumarate | 5.0 |
| 6 | Iron Dextran | 8.8 |

Thus, it is clear that ferrous sulphate is the cheapest, ferrous gluconate and succinate are about two to four times more costly than ferrous sulphate, whereas, ferrous fumarate is about five times more costly than ferrous sulphate. Parenteral treatment is still considerably more expensive than oral treatment. It would be clearly uneconomic to substitute an expensive iron compound just to eliminate the side effects that involve some of the patients. In our country cost of treatment is naturally a deciding factor.

Only two patients treated with ferrous fumarate complained of mild constipation, whereas, ferrous sulphate was surprisingly well tolerated by all the patients. Iron has got many incompabilities, which might be responsible for side effects. I have prescribed only iron and found minimum or no side effects.

Thus, the absence of substantial side effects in almost all the cases of the present series deserves special mention. Interestingly, this important finding is in accordance with only a few of the workers (Parekh et al., 1958; Rajsuriya et al., 1961). The earlier reports of a very high incidence of gastro-intestinal intolerance to oral iron in about one-third of all cases(Benstead and Theobald, 1952) were due to the fact that trials were

carried out on pregnant women. Intolerance is commoner in pregnancy than at any other time (O'Sullivan et al., 1955) who, like the present series, excluded pregnant women.

Although the lower incidence of intolerance in patients receiving the organic iron preparations is an advantage, nevertheless, the variations in cost and the greater expense of many of these preparations make their routine prescription at present a matter for thought.

Kerr and Davidson (1958) found no difference in the incidence of side-effects between various iron compounds and control tablets. They gave ferrous sulphate, ferrous gluconate, ferrous succinate and ferrous calcium citrate in daily doses containing 105 mg. of elemental iron and "known" and "unknown" control pills containing lactose, to 93 healthy young women in a "double blind" trial, that is neither doctor nor patient knew which of the preparations had been given to which patient, except in the case of the "known " control pills. None of the four iron preparations was found to induce toxic effects more frequently than did the "unknown" control lactose pills. All the preparations (including "unknown" control pills) gave an incidence of gastro-intestinal symptoms between 15 to 20 per cent, whereas, the "known" control pills produced symptom in less than 2 per cent of the

patients.[77] They, therefore, concluded that the side-effects were psychological in origin. Again, Edgar and Rice (1956) found that the usual high rate of intolerance to ferrous sulphate reported in pregnant patients was reduced to less than 5 per cent when the product was given in the form of white tablet instead of the well-known green form. Thus, the decreased incidence of such side-effects when the colour of the tablets is changed, lends support to the above hypothesis.

The remarkable absence of gastro-intestinal side effects in the patients of the present series is probably due to the fact that these patients came from poor economic levels and had never heard of oral iron and its side effects. Gatenby's (1959) claim that intolerance is more common in the severe degree of anaemia has certainly not been substantiated by the present findings.

Failure to respond to haematinic agents may indicate that anaemia is not due to iron deficiency, that complicating factor is interfering with the ability of bone marrow to respond, that there is continuous active blood loss, or that there is impaired absorption of iron. Thus, it is important in all cases to search for the underlying cause of anaemia. As mentioned earlier, no patient having been once treated with ferrous sulphate or ferrous fumarate has

been excluded from this series on the grounds of failure to respond to iron therapy or toxic effects.

## RESULTS

### Therapeutic Response :

The administration of 180 mg. of elemental iron per day as ferrous sulphate or ferrous fumarate has proved to be therapeutically effective in all the patients included in the study.

Parekh and Rane (1958) judged the efficacy of the drug on the basis of the mean daily rise of haemoglobin. It was considered 'very good' when the haemoglobin rise was over 1.1 per cent per day, 'good' when the response was between 0.5 to 1.1 per cent per day, and 'poor' when it was less than 0.5 per cent per day.[95] The patients in the present series have shown either 'very good' or 'good' therapeutic response. The majority of the patients showed satisfactory response both clinically and haematologically. In all the patients, there was symptomatic relief too. The patient's own appreciation of improvement was gratifying.

The rise in haemoglobin is shown as a graph in which the average weekly increase from the initial values obtained in all the patients is plotted against the number of weeks during which the treatment was given. The graph method is probably the more accurate and informative method

of assessing the therapeutic value of a compound because the influence of the duration of treatment on the results can be better appreciated.

The percentage utilisation of iron as recorded in table 27 was calculated as per Franklin's formula,[49] which is based on the following considerations.

The total rise in haemoglobin (grams per 100 ml.) during the therapy was multiplied by 50 to obtain a rise in haemoglobin per 5000 ml. of blood(which is taken as an average normal blood volume). The product was then multiplied by 0.0033 (that portion of haemoglobin molecule which is composed of iron). The result so obtained was divided by the total amount of elemental iron in grams given during the therapy, and finally multiplied by 100 to obtain the percentage utilisation.

This figure for percentage utilisation of iron does not, therefore, include any iron which entered into formation of non-haemoglobin body constituents or was added to body stores. Although such values for percentage utilisation of iron are admittedly subjected to error due to the assumption of 5000 ml. as the average adult blood volume, they serve to approximate the degree to which the iron of ferrous sulphate or ferrous fumarate is utilised for haemoglobin regeneration. These values indicate that

the iron of ferrous sulphate is utilised more markedly and rapidly as compared to that of ferrous fumarate (Table No. 27 ).

Ferrous sulphate gives greater and quicker haemoglobin rise than ferrous fumarate. This is true for all other haematological responses (Table 25 to 34 ). Moreover, different investigations show that the maximum, minimum and mean haematological responses are also greater after ferrous sulphate administration than after ferrous fumarate administration (Table 51). Thus, it is clear that ferrous sulphate is a more efficient (and equally tolerable) therapeutic agent than ferrous fumarate, its special merit being the cheapness.

The overall impression of the results of ferrotherapy in iron deficiency anaemia cases indicates that iron therapy leads to improvement in size and shape of R.B.Cs as well as filling of haemoglobin, but does not markedly increase the number of R.B.Cs. It also suggests that, in uncomplicated cases of iron deficiency anaemia, M.C.H.C. rise is in proportion to the Haemoglobin rise.

The maximum, minimum and mean haematological responses obtained in the two series (Series A and Series B) are given in the following table for easy comparison;-

TABLE NO. 52

MINIMUM, MAXIMUM & MEAN HAEMATOLOGICAL RESPONSES

| Sr. No. | Investigations | Rise | Fe - S | Fe - F |
|---|---|---|---|---|
| 1. | Hb - gm% rise per week (All values of Hb in gm%) | Minimum | 0.723 | 0.240 |
| | | Maximum | 1.547 | 0.920 |
| | | Mean | 1.207 | 0.666 |
| 2 | Hb - percentage rise per day (All values of Hb in %age) | Minimum | 0.714 | 0.238 |
| | | Maximum | 2.143 | 0.905 |
| | | Mean | 1.290 | 0.657 |
| 3. | Fe - percentage utilization (All values in percentage) | Minimum | 9.472 | 3.142 |
| | | Maximum | 20.25 | 12.04 |
| | | Mean | 15.81 | 8.730 |
| 4. | R.B.C. Rise per week (All values in m/cmm) | Minimum | 0.067 | 0.020 |
| | | Maximum | 0.293 | 0.203 |
| | | Mean | 0.152 | 0.096 |
| 5. | Colour Index -rise per week (All values in percentage) | Minimum | 0.0461 | 0.0122 |
| | | Maximum | 0.1163 | 0.0853 |
| | | Mean | 0.0928 | 0.0531 |
| 6. | P.C.V. rise per week (All values in percentage) | Minimum | 1.333 | 0.667 |
| | | Maximum | 3.667 | 2.333 |
| | | Mean | 2.616 | 1.749 |

| Sr. No. | Investigation | Rise | Fe - S | Fe - F |
|---|---|---|---|---|
| 7. | M.C.V. rise per week (All values in c u) | Minimum | 1.113 | 0.493 |
| | | Maximum | 7.393 | 6.167 |
| | | Mean | 4.371 | 3.211 |
| 8. | M.C.H. rise per week (All values in u u g) | Minimum | 1.280 | 0.230 |
| | | Maximum | 4.220 | 2.570 |
| | | Mean | 2.846 | 1.567 |
| 9. | M.C.H.C. Rise per week (All values in percentage) | Minimum | 1.073 | 0.117 |
| | | Maximum | 3.160 | 2.013 |
| | | Mean | 2.296 | 1.140 |
| 10 | Reticulocyte Response (All valuesin percentage) | Minimum | 3.6 | 2.3 |
| | | Maximum | 11.9 | 8.1 |
| | | Mean | 8.52 | 5.51 |
| 11 | Percentage of cases showing peak of Reticulocyte response on : | 11th day | 30 | 15 |
| | | 13th day | 55 | 50 |
| | | 15th day | 15 | 35 |

This trial was designed to ascertain whether ferrous fumarate was superior to ferrous sulphate. It is found that it is no better than the tried, tested and time-honoured ferrous sulphate as regards therapeutic efficacy and freedom from undesirable side effects. On the contrary, ferrous sulphate being much cheaper and more efficient than ferrous fumarate, therefore, still remains the drug of choice for routine treatment of iron deficiency anaemia.

Electrocardiographic Results :

The electrocardiographic study reveals that cases of anaemia can show important electrocardiographic changes like sinus tachycardia, low voltage, abnormal ST segment and abnormal T wave, and that such abnormalities may resemble the cardiac infarction pattern or coronary insufficiency pattern. These findings are in agreement with those of other workers (Wood, 1950; Shah, 1953).

About 35 per cent of the cases of the present series showed slight to moderate enlargement of heart. On treatment of anaemia, heart size started returning to normal. Enlarged heart was usually associated with abnormal electrocardiogram, and greater the enlargement, more marked were the electrocardiographic changes. It may be added, however, that the heart size and electrocardiogram may be normal in cases of severe anaemia.

None of the patients included in this series were obese. Thus, obesity is ruled out as the cause of the above-mentioned electrocardiographic changes. Kadish[75,76] reported that electrocardiographic abnormality varies with the patient's weight. These consisted of T wave changes, including inversion in leads I and $V_6$ in all but one case. No ST segment changes were reported. The co-relation of abnormalities with loss and gain of weight in these patients is suggestive of increased cardiac strain with obesity.

Serum Iron In Normal Subjects :

The normal range of serum iron as observed in this study is 150 to 250 (average 196) mcg. per 100 ml. in males, and 100 to 200 (average 148) mcg. per 100 ml. in females (Table 38 and 39 ).

There is an overlapping in the range of serum iron values of normal males and normal females, but there is a clear difference in the mean values, that is, the serum iron values in normal females are significantly lower than those in normal males. Similar observations have been reported by many workers (Vahlquist, 1941; Powell, 1944; Laurell, 1947; Pirrie, 1952 and Davies et al., 1952), although some workers (Cartwright and Wintrobe, 1949; Stengle and Schade, 1957) have failed to observe any significant difference in the two sexes. In most of the other physiological and biochemical data, a significant difference in two sexes is found and there is no reason why one

should not get the same in serum iron.

Thus, most of the workers found that the mean value for serum iron in normal males is higher than in normal females. The results of the present study are in agreement with this.

The cause for low serum iron values in females is not known. According to McCance (1936) and Powell(1944), menstrual blood loss was sufficient to explain the difference. On the other hand, Gordon and Cheripper (1947) believed that the difference between the two sexes in serum iron values seen in human being may be of hormonal nature. Vahlquist (1950) failed to observe any significant rise in serum iron in 22 normal adult females to whom he administered 300 to 336 mg. of ferrous iron daily for a period of 8 weeks to compensate for the menstrual blood loss. If it be admitted that the rise in haematologic values takes place in males after puberty, whereas the values remain the same in females. It would appear that endocrine influences may be responsible for the difference in serum iron values in the two sexes (Gupta et al.,1959).

Serum Iron in Anaemic Subjects :

The range of serum iron in cases of iron deficiency anaemia as observed in the present study is 30 to 90

(average 61) mcg. per 100 ml. in males and 27-70 (average 46) mcg. per 100 ml. in females (Table 40 and 41 ).

There is an overlapping in the range of serum iron values of anaemic males and anaemic females but there is a difference in the mean values. Thus, the serum iron -values in male patients of iron deficiency anaemia are found to be higher than those in female patients. These results definitely show that the range and mean of serum iron values in iron deficiency anaemia are lower than those found in normal subjects.

Serum Iron Response :

The serum iron level rises markedly after the oral ingestion of 180 mg. of ferrous sulphate or ferrous fumarate (Table 44 ), indicating that both these compounds are well absorbed. However, the rise in serum iron after ferrous fumarate administration is slightly more as compared to that after ferrous sulphate administration. Thus, there was a good response to the oral administration of these iron compounds. No patient has been excluded from this series on the grounds of poor serum iron response to the oral administration of iron.

The fall in serum iron (8 hours sample) is remarkably more in case of ferrous sulphate as compared to that in case of ferrous fumarate (Table 45 ). The excretion

of iron in urine is not increased by oral administration of large doses of the metal.[26] Thus, the urinary excretion is minimum and it is so probably because the circulating iron is always bound to protein. Faecal excretion is out of question when one considers the serum iron. Thus, the urinary and faecal excretion of iron is ruled out. Hence, the serum iron can either go to stores or can be utilised.

The difference in serum iron response obtained after ferrous sulphate administration and that obtained after ferrous fumarate administration has been clearly brought out in the colour intensities shown in Photograph No.26 (page 292).

Thus, the sulphate as well as the fumarate iron are well absorbed but the fall in fumarate iron is poor. Marked fall in sulphate iron is explained by greater haemoglobin rise and greater percentage utilisation. This is not so in case of fumarate iron. Therefore, it is probable that fumarate iron which is not yet fully tried and tested, might be forming loose complex with the protein, and hence can be easily and slowly excreted in urine. This is supported by good rise (indicated by high serum iron values after 4 hours of administration of iron), associated with poor therapeutic response. This can be confirmed by simultaneous serum iron as well as

urine iron estimation which was, of course, out of scope so far as the present study is concerned.

Serum iron response in two sexes (male and female patients) did not reveal any significant difference (Table 46 ), but serum iron response on 1st and 21st day of treatment did reveal significant difference (Table 43 ). The response is more marked on 1st day of treatment as compared to that on 21st day. Thus, the response is more marked in severely anaemic (untreated) patients than that in patients treated for three weeks. This is true in case of [illegible] sulphate as well as fumarate iron.

Fasting values obtained on 21st day of treatment are higher than those obtained on 1st day of treatment. This is because fasting samples of 21st day are same as approximately 18 hours samples of the previous day (20th day). Fasting values on 21st day in case of ferrous fumarate are higher than the corresponding values [illegible] in case of ferrous sulphate. This finding is in accordance with the poor fall (8 hours samples) of fumarate iron on first day of treatment (vide supra).

The maximum, minimum and mean serum iron values of the different blood samples collected on 1st and 21st day of treatment are given in the following tables for [illegible] easy comparison.

TABLE NO. 53

MINIMUM, MAXIMUM & MEAN SEFUM IRON VALUES
(All values in r per 100 ml.)

| Sr. No. | Sample | Serum Iron values | First day of treatment | | Last day of treatment | |
|---|---|---|---|---|---|---|
| | | | Fe - S | Fe - F | Fe - S | Fe - F |
| 1. | T1 | Minimum | 27.00 | 30.00 | 41.43 | 97.14 |
| | | Maximum | 90.00 | 90.00 | 130.00 | 184.3 |
| | | Mean | 52.49 | 54.86 | 88.21 | 151.9 |
| 2. | T2 | Minimum | 561.4 | 694.3 | 481.4 | 458.6 |
| | | Maximum | 985.7 | 1043. | 891.4 | 910.0 |
| | | Mean | 764.8 | 868.1 | 657.0 | 750.6 |
| 3 | T3 | Minimum | 145.7 | 585.7 | 158.6 | 405.7 |
| | | Maximum | 502.8 | 957.1 | 552.8 | 790.0 |
| | | Mean | 255.2 | 707.5 | 305.27 | 636.1 |
| 4 | Rise in serum levels | Minimum | 517.1 | 662.8 | 381.4 | 361.4 |
| | | Maximum | 928.5 | 973.0 | 768.6 | 762.8 |
| | | Mean | 712.3 | 813.2 | 568.8 | 599.8 |
| 5. | Fall in serum Iron levels | Minimum | 414.3 | 85.90 | 181.4 | 52.90 |
| | | Maximum | 585.7 | 296.6 | 500.0 | 195.7 |
| | | Mean | 509.6 | 194.2 | 351.7 | 109.5 |

TABLE NO. 54

MINIMUM, MAXIMUM AND MEAN SERUM IRON VALUES
(All values in r per 100 ml)

| Sr. No. | Sample | Serum Iron Values | Fe - S | | Fe - F | |
|---|---|---|---|---|---|---|
| | | | First day | Last day | First day | Last day |
| 1. | T1 | Minimum | 27.00 | 41.43 | 30.00 | 97.14 |
| | | Maximum | 90.00 | 130.0 | 90.00 | 184.3 |
| | | Mean | 52.49 | 88.21 | 54.86 | 151.9 |
| 2. | T2 | Minimum | 561.4 | 481.4 | 694.3 | 458.6 |
| | | Maximum | 985.7 | 891.4 | 1043 | 910.0 |
| | | Mean | 764.8 | 657.0 | 868.1 | 750.6 |
| 3. | T3 | Minimum | 145.7 | 158.6 | 585.7 | 405.7 |
| | | Maximum | 502.8 | 552.8 | 957.1 | 790.0 |
| | | Mean | 255.2 | 305.2 | 707.55 | 636.1 |
| 4. | Rise in Serum iron levels | Minimum | 517.1 | 381.4 | 662.8 | 361.4 |
| | | Maximum | 928.5 | 768.6 | 973.0 | 762.8 |
| | | Mean | 712.3 | 568.8 | 813.2 | 599.8 |
| 5. | Fall in serum Iron levels | Minimum | 414.3 | 181.4 | 85.90 | 52.90 |
| | | Maximum | 585.7 | 500.0 | 296.6 | 195.7 |
| | | Mean | 509.6 | 351.7 | 194.2 | 109.5 |

Stability of the Colour :

An attempt has been made, for the first time in this study, to establish the stability of the colour for spectrophotometric readings. A special procedure has been adopted for this purpose and the same has been fully described (Page 296) as well as discussed (Page 304) in Chapter 19.

On comparing the serum iron values obtained from "Immediate Readings" and thos obtained from "readings after 24 hours" (Table 42 , Chapter 27), it has been found that the two values of serum iron compare well and that the difference between the two values falls within the experimental error ( ± 5 per cent).

All the different investigators have so far suggested to take the readings within 1/2 hour of the development of colour. This short time-limit is the greatest handicap which has been successfully overcome by the present establishment of the stability of the colour for spectrophotometric readings.

Thus, the results of the present study show that the colour is stable for a minimum period of 24 hours as against the maximum time-limit of 1/2 hour given so far.

## COMPARISON WITH OTHER WORKERS

The following four tables show the comparative results as regards the Haemoglobin rise, the serum iron values in normal subjects and the serum iron values in anaemic subjects.

### Haemoglobin Response :

The results of the present study reveal that the haemoglobin rise is 1.29 per cent per day after ferrous sulphate administration. This figure compares well with that found by O'Sullivan et al. (1955), and that the percentage utilisation is 15.8 which compares well with that obtained by Witts (1936), and O'Sullivan et al. (1955). The haemoglobin rise obtained after ferrous fumarate administration in this study is 0.657 per cent per day and the percentage utilisation is 8.05. This compares well with that found by Drug Council (1959).

The per-centage rise of haemoglobin and the percentage utilisation of iron as obtained by different workers are shown in the following table.[14,92,105]

TABLE NO. 55

COMPARISON OF ORAL IRON COMPOUNDS BY DIFFERENT WORKERS

| Iron Compound | Investigator | Daily dose of Elemental Iron in mg. | Daily Rise of Hb.in percent. | Percentage Utilisation |
|---|---|---|---|---|
| Ferrous Sulphate | Witts (1936) | 180 | 1.00 | 14.8 |
| | O'Sullivan et al. (1955) | 200 | 1.20 | 14.4 |
| | Rajsuriya et al. (1961) | 180 | 1.82 | 25.3 |
| | Present series | 180 | 1.29 | 15.8 |
| Ferrous Fumarate | Drug Council(1959) | 195 | 0.666 | 8.05 |
| | Berenbaum et al. (1960) | 195 | 0.944 | 12.0 |
| | Rajsuriya et al. (1961) | 195 | 1.930 | 24.7 |
| | Present series | 180 | 0.657 | 8.73 |

Serum Iron in Normal Subjects :

Different workers have found different figures for normal serum iron values. Nevertheless, most of them agree that the mean value for serum iron in normal males is higher than that in normal females.

The serum iron values obtained here for normal subjects need little comment. The mean for 20 normal males (Indian subjects) is 196 mcg. per 100 ml. which is in good comparison with the figures (176 mcg. per 100 ml.) obtained (in Indian subjects) by Mody, Dixit, Parekh, Jhala and Ramsarma (1956). The lowest quoted mean for normal males is 103 mcg. per 100 ml. (Pirrie, 1952), while the highest quoted mean is 176 mcg. per 100 ml. (Mody et al., 1956). The mean for 20 normal females (Indian subjects) is 148 mcg. per 100 ml. which is again in good comparison with the figures (135 mcg. per 100 ml) obtained by Vanotti and Delachaux (1949). The lowest quoted mean for normal females is 88 mcg. per 100 ml. (Albers, 1941), while the highest quoted mean is 135 mcg. per 100 ml. (Vanotti and Delachaux, 1949). Moreover, the overall range of serum iron values obtained here in normal subjects (males as well as females) which is 100 to 250 mcg. per 100 ml., is in good comparison with that found by Smith (1952) viz. 60 to 220 mcg. per 100 ml.[135]

The results of such investigations undertaken by different workers in India and abroad show close proximity in some and wide divergence in others (Table 56 and 57 ). Thus, there are certain discrepancies between the results obtained by different investigators. Such discrepancies may be due to multitude techniques employed. In this context, it is important to note that there was a frequent disagreement between the results obtained on a single specimen by different methods (Peters et al., 1956). But this is not all, because different types of subjects selected, and above all, the uncontrolled factors affecting the serum iron values also may be highly responsible for the diff discrepancies in the results obtained by different workers.

It is difficult to understand why the earlier results of Moore, Arrowsmith, Quilligan and Read (1937) were consistently lower than those of Vahlquist (1941) and the successors. Low serum iron values obtained by other workers might possibly be due to the concomittant intake (either as prescribed therapy or as self-medication) of hypoferremic drugs like salicylates which are so commonly available and so widely used for the relief of common symptoms like pain. The values obtained in the present study are slightly higher perhaps because of the fact that all the factors affecting the serum iron values are considered and possibly controlled. These higher values

definitely indicate that the modified method adopted here for serum iron estimation ensures complete extraction of iron, whilst the associated consistent results reflect in the utility and reliability of the method for serum iron estimation which is much modified during the course of the present study.

It is note-worthy that some workers have carried out the serum iron estimations in normal males as well as in normal females. Evidently, this facilitates the comparison of normal serum iron values in the two sexes since the variations due to the type of subjects selected and those due to the technique employed for serum iron estimation are minimised. Thus, the inclusion of subjects belonging to either sex in a particular set of experiment, as has been done in this study, is of greater importance. Contrary to this, some workers have carried out the serum iron estimations in only either of the two sexes.

The following two tables show the range & mean and mean respectively of the serum iron values[54,72,89] in normal subjects as obtained by different investigators.It is clear from the tables that very little work has been done in India and hence the values in Indian subjects are hardly available for comparison.

Serum Iron in Anaemic Subjects :

A careful review of the literature on serum iron reveals that much less work has been carried out on serum iron estimation in anaemic subjects and serum iron response to iron administration. Accordingly, only one worker's (Powell, 1944) values are available in the literature for comparison. The serum iron values in anaemic subjects obtained during the present study are in close agreement with those found by Powell.[100] This is shown in the following table :

TABLE NO. 58

SERUM IRON VALUES IN ANAEMIC SUBJECTS

| Investigator | Normal subjects | | | Anaemic subjects | | |
|---|---|---|---|---|---|---|
| | No. of subjects | Serum iron in r% | | No. of subjects | Serum iron in r% | |
| | | Range | Mean | | Range | Mean |
| Powell | 70 | — | 130 | 30 | 25-90 | 47 |
| Present Series | 40 | 100-250 | 172 | 40 | 27-90 | 54 |

The normal values obtained by the same workers are also given in the table for comparison.

Stability of the Colour :

A careful review of the literature on serum iron clearly indicates that no investigator has so far attempted or undertaken this aspect of serum iron estimation to establish the stability of the colour for spectrophotometric readings. It is, therefore, not possible to compare the results with other workers.