DISCUSSION
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Iron deficiency anemia is one of the important causes of considerable illhealth and morbidity in our country. While its various facets have been under study and detailed reviews are available in the literature, studies on body iron stores in anemias before, during and after treatment are scanty, especially in this country. To have a quantitative idea of body stores of iron before and during replacement therapy would seem to be a logical and desirable information which has promise of clinical application. No such information is available in published material from India.

The present study was therefore undertaken to investigate this aspect of the problem and in the process an indirect method of quantitation was used, not described previously. Only the first twelve days of parenteral iron therapy in the treatment of anemia of iron deficiency have been investigated in the present study and no doubt work will have to be extended to cover further periods of therapy.

The observations described in the previous chapter involved establishment of hematological parameters in cases of iron deficiency anemia, observations on
haemoglobin rise, and morphological and histochemical appearances of bone marrow preparations and liver biopsy sections. Calculations used and analysis of results have already been described and this aspect of the study is being presented in some detail in the succeeding pages.

Iron staining of bone marrow smears has been most widely used as a means of assessing status of storage iron in man (Rath and Finch, 1948; Masshoff and Grener, 1951; Hutchison, 1953; Ellis, Jenson and Westerman, 1964; Beatlar, Robson and Butterweiser, 1958 and Nissen and Olson, 1968). And yet it has, remained simply a qualitative technique. Although the system of grading (into 0-4+) does impart to it the merit of semiquantitation, and has proved in itself as a valuable aid in diagnostic work, yet it does not indicate measurable amounts of iron deposited in the stores.

A large number of methods have recently become available which can precisely measure the amount of iron present in the stores. Chemical estimation of non-haem iron and measurements by combined radioisotopic dilution and chelation methods (Fielding, 1967; Balcerzak, Westerman, and Tayler, 1968) can be cited as some of these. But these
methods are too elaborate, tedious, time consuming and outside the scope of routine diagnostic laboratories, at least in our country. In the present study, an attempt has, therefore, been made to add some more precision to the quantitative aspects of the histochemical method of the assessment of storage iron i.e. iron staining of bone marrow smears. One method to do this would be to calculate the amount of iron in the stores which would be indicated when stainable iron reappears in the bone marrow as a result of iron therapy.

In the following description, the data presented in the previous chapter is discussed in this regard. This is followed by a discussion on the validity of the method adopted in the present study to gain such information. Some ancillary observations of the study are also discussed at the end.
Only the patients with complete absence of stainable iron in the bone marrow smears (grade 0) were taken in this study. The reappearance of marrow stainable iron, upon exogenous iron administration in these subjects, would rationally be related to the amount getting deposited in the stores.

In the present study a variable amount of iron dextran complex (imferon) was administered parenterally (intramuscularly) as is shown in Table VII. From different subjects it varied from 0.60 to 1.2 gm. over a period of 6 to 12 days from start of treatment.

After accounting for the amount of iron remaining unabsorbed at the injection site and for the amount out of the absorbed iron getting utilised for new haemoglobin synthesis during the 12 days period of study, the amount presumed to have reached the stores was calculated to vary from 161.9 mg. to 452.4 mg. This was the amount calculated to be deposited in the body stores, as reflected in the bone marrow during the 6th to 12th day period of the schedule employed. The
amount depicts a wide range of values which has only indirect relevance to the amount of iron which must be presumed as the minimum replenishment of stores at the time when either it becomes stainable or indirectly when haemoglobin rise begins to reflect the response to treatment.

By studying different groups of subjects on different post therapy days it was observed that iron was first demonstrated in the bone marrow deposits by the prussian blue reaction on the 6th day. Therefore, the amount of iron in the storage sites after 6 days of parenteral iron therapy could be considered as the minimum amount which must be present so as to make it demonstrable by histochemical methods. At this time the amount of iron utilization is also the minimum (mean 37.23 mg.), and so is the extent of haemoglobin rise (mean 0.3 gm.) - See Table IX and VII.

Table I reflects the calculations for the study period, where it is shown that for the 6th day observation, the calculated amount of iron presumed to be deposited in the storage sites was 352.6±31.3 mg. and that this could be regarded as the smallest amount that must be deposited in the stores as a result of iron dextran administration at the time when iron is first demonstrable in the bone marrow aspirates by histochemical
methods, assuming that the absorption from the injection site is to the extent of 65% of the amount injected.

The rate of absorption of iron dextran from injection site is really valid for the 7-10 days period (Grimes and Hutt, 1957 and Evans and Ramsey, 1957) and the amount of marrow injection on the one hand and 7th and 10th day values on the other hand are not statistically significant, a more balanced approach to this approximate quantitation would be to find an average figure for the values obtained for the 6th, 7th and 10th days and consider this mean figure to represent the amount of iron which is deposited in the body stores at the time when iron is readily stainable for the "first" time after the start of iron dextran therapy and the response in haemoglobin rise has "just" begun. This amount was found to be 371.3 mg. ± 40.3 mg.

No such work has been reported in literature. The results obtained in the present study therefore could not be compared with any published account. Nor, has the same
problem where in the amount of storage could be predicted by its stainability - been studied by any different procedure. However, before the results of the present study can be accepted, even for indirect connotations, the validity of the procedure adopted here should be discussed.
VALIDITY OF THE PRESENT PROCEDURE
AND CALCULATIONS USED

The variabilities which could influence the results obtained in the present study are:

- The total amount of injected iron in different subjects.
- The duration of the study and the day of reappearance of stainable iron in bone marrow smears.
- The initial haemoglobin levels of the subjects.
- The quantum of haemoglobin rise in different subjects.
- Variations in the proportion of injected iron remaining unabsorbed at the injection site.

Apart from these variables, a serious consideration has to be given to the fact that the stainable iron demonstrated in the study is the exogenously administered iron-dextra-complex rather than the endogenous haemosiderin.
iron which represents the natural iron stores. And therefore the application of these results to the situation in vivo shall need clarifications and reservations.

As far as the various variables enumerated above are concerned, reference should be made to Figure VII to X shown in the previous chapter. These figures show the relationship between the amount of iron calculated to have reached the storage sites on the one hand and (i) age and sex, (ii) initial degree of anaemia, (iii) quantum of rise in haemoglobin%, and (iv) the day of appearance of iron in the bone marrow smears, on the other hand. It is amply clear that there is no correlation between these parameters, except that the quantum of rise in haemoglobin percentage is inversely proportional to the amount remaining in the storage sites but as described earlier even this does not alter the amount remaining in the storage sites between 6th post therapy day and 10th post therapy day (See observation page 95-96), which means that these factors do not influence the amount of iron reaching the marrow iron stores under the conditions of the present study. It has been reported that the stainability and the utilisation of iron dextran aggregates is comparable with the haemosiderin iron aggregates in the bone
narrow smears and the other organs of iron stores
(martin et al, 1955).

In case of single total dose infusion of iron dextran complex, it has been reported that this form of iron from the plasma is cleared exponentially into the pools, with a continuous feed back between these pools (kumar et al, 1974). As the internal cycle of iron metabolism is virtually a closed cycle, the injected iron gaining entry into the body is not excreted outside in more than negligible amounts. And, if it is partitioned into more than one pool, these could only be the plasma pool and the storage pool, from both of which it may be available for heme globin synthesis. These are precisely the pools into which the endogenously released iron is also distributed.

As far as the variations in the absorbability of injected iron from the injected site are concerned, the calculations made in the present study are based entirely on the findings published by other workers (grimes and hutt, 1957 and evans and ramsay, 1957). Based upon these studies, a proportion of 65% absorption has been taken as valid for calculations made in this study.
Whether the stainability of endogenously administered iron dextran complex in the bone marrow smears should be considered in equivalence with the endogenously available iron stores as far the objective of the present study is concerned, needs serious consideration. Here again, support is taken from the published reports about the metabolism and handling of iron dextran complex in the body (Martin et al., 1955).

In view that the various variables enumerated above do not influence the results of the present study and also after considering that the technique is valid, one more aspect still remains to be discussed before the results are accepted for summation and conclusions. In the calculations made in the present study, no consideration has been made of the dietary iron which after absorption would also be contributing towards new haemoglobin synthesis, during the period of the study. Although attempt was made to keep these patients on as much a low iron content diet as possible, yet the diet given cannot be considered to be iron free.

It has also been shown that patterns of plasma clearance of iron dextran complex and of the tracer doses of radio-active iron (which represents the clearance patterns of
endogenously available iron) are similar, only the quantitative aspects of their clearance are different (Kumar, 1974).

Therefore, it appears that if the patterns of pool exchanges and the stainability of aggregates of exogenous iron dextran complex and the endogenous released iron are similar (even if not the same) the results of the present study can be assumed to mimic the conditions in vivo to a rational degree of faithfulness.

However, it is possible that the results obtained in this study, indicate slightly lower than the actual amount of iron delivered to the stores, because somewhat lesser than the calculated values would have been actually utilised for haemoglobin synthesis.

With this proviso, however, the present study does provide the conclusion that when the iron first becomes "clearly" stainable in the bone marrow, a minimum of 37.3 ± 40.3 mg. of iron is already deposited in the body stores as a result of therapy. This occurs between the 6th to 10th day of start of such treatment as described in the schedule of iron dextran therapy employed in the present study.
It can also be concluded that when the stainable iron is demonstrable in the reticuloendothelial cells of bone marrow smears, it is not necessarily stainable in the kupffer cells of the liver. It perhaps would need greater amounts of iron to be administered over longer period of time or in other words, greater replenishment of depleted iron stores in the body before being histochemically demonstrable in the liver. No observations have been made, in the study, upon other reticuloendothelial organs like spleen, lymph node etc.

An indirect application of the results of this study may find expression in estimating the total minimum amount of iron required to be administered in treating cases of iron deficiency anemia with depleted body stores.