Apparent Folate Deficiency in Iron-Deficiency Anaemia

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SUMMARY. Examination of 50 patients with iron-deficient hypochromic anaemia showed evidence suggesting a high incidence of folate depletion. The peripheral blood films of 44 patients (88%) showed more than 3% of neutrophils with five nuclear lobes, 35 patients (70%) had a high mean neutrophil lobe count, 29 patients (40%) had hypersegmented neutrophils and 19 patients (38%) had giant metamyelocytes in the bone marrow. The serum folate was below 3 ng/ml in 18 patients and 3-6 ng/ml in 18. Red-cell folate was subnormal in 15%, and 45% had a positive Ficultest.

Correlation between various tests for folate deficiency was not found, apart from a correlation between red-cell folate levels and morphological changes in the neutrophils. The haemoglobin rise following intravenous iron therapy was smaller when the serum folate level was low. There was probably a similar relationship to the red-cell folate level, but the numbers tested were small. The presence of neutrophil multilobing in the peripheral blood film and giant metamyelocytes in the bone marrow did not influence the response to iron therapy, neither did an abnormal Figlu excretion.

Following intravenous iron therapy, both neutrophil multilobing and marrow giant metamyelocytes were significantly reduced in number. This therapy did not significantly alter Figlu excretion measured 6 weeks after treatment, but both serum and red-cell folate levels fell. Intravenous iron therapy did not produce any significant changes in the serum vitamin B₁₂ levels. The part that iron deficiency itself may play in causing apparent folate depletion is discussed.

The morphological changes in peripheral blood and bone marrow due to iron deficiency may mask evidence of coexisting folate and vitamin B₁₂ deficiency (Pedersen et al., 1957; Taker, 1959). Further work has suggested that there is a more fundamental association between iron deficiency and the morphological and biochemical changes interpreted as evidence of folate deficiency. Thus iron-deficient rats on an adequate folate intake showed an increase in the urinary excretion of formiminoglutamic acid (Figlu), with a reduction in liver formiminotransferase, a fall in serum folate, and megaloblastic changes in the bone marrow (Vitale et al., 1965a, b, 1966). These authors suggested that the enzyme formiminotransferase is iron-dependent and that iron deficiency can interfere with folate metabolism by

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reducing the activity of this enzyme. Burns & Spray (1969), however, found no change in either liver formiminotransferase activity or liver and serum folic acid activity in rats fed an iron deficient diet.

In 1962 Chanarin, Bennett & Berry noted an abnormal urinary excretion of histidine derivatives in iron-deficiency anaemia and suggested that this was associated with an abnormally rapid folic acid clearance in this condition. Hansen (1964) found giant monocyte or hypersegmented neutrophils in 38% of a series of patients with hypochromic anaemia, with a tendency to low serum folate levels but high whole blood folate levels, and all the patients he tested for Figlu excretion showed a normal result.

In pregnant women the highest incidence of megaloblastic change, raised Figlu excretion and reduced serum folate was found in the iron-deficient group; iron therapy reduced the incidence of these changes (Chanarin et al., 1965; Chanarin, 1967). Hoffbrand et al. (1967) found lower mean serum folate and vitamin B12 levels in post-gastrectomy patients with iron deficiency compared to a similar group without iron deficiency. However, in each of 17 patients with post-gastrectomy anaemia and evidence of iron and folate deficiency, the haemoglobin level returned to normal with iron therapy alone (Hines et al., 1967). In aneupotent patients the response of iron-deficiency anaemia to intravenous iron-dextran may be influenced by coexisting folate deficiency (Basu, 1965; Bonnar, 1965). Metz et al. (1962) obtained a higher mean haemoglobin rise in patients with post-partum anaemia treated with iron-dextran and folic acid compared with those treated with iron alone.

The present investigation was designed firstly to look for evidence of folate deficiency in a group of patients with unequivocal and apparently uncomplicated iron-deficiency anaemia, secondly to assess the effect of iron therapy on the changes suggesting folate depletion, and thirdly to study the influence of this apparent folate deficiency on the response to iron therapy.

**MATERIALS AND METHODS**

**Subjects Studied**

For the purpose of this study 50 patients with iron-deficiency anaemia were selected. No patient had a haemoglobin level greater than 10.5 g/100 ml, and all showed marked hypochromasia of the red cells in the peripheral blood and a complete absence of stainable iron in bone marrow fragments. Each patient was thoroughly investigated to determine the cause of the iron deficiency. The final clinical diagnoses were: partial gastrectomy, 10 patients; duodenal ulcer, 5; hiatus hernia, 5; gastric ulcer, 4; menorrhagia, 4; rectal bleeding from haemorrhoids or diverticular disease of the colon, 3; ulcerative colitis, 2; blood loss associated with ingestion of salicylates, 2; adult coeliac disease, 1; partial gastrectomy plus peptic oesophagitis, 1; partial gastrectomy plus epistaxes, 1; partial gastrectomy plus menorrhagia, 1; cause undetermined, 11. There was no clinical evidence of gastrointestinal blood loss at the time of investigation and faecal occult blood tests were negative. In addition to the clinical diagnoses a detailed dietary history showed deficient iron intake in 34 of these patients. In these the estimated dietary iron intake was less than the 10 mg/day in men and post-menopausal females, and less than the 15 mg/day in menstruating females recommended by the National Research Council of the U.S.A. (1964). None of the patients had received iron or folic-acid supplements and none had taken any drugs known to interfere with folate metabolism.
Folate Deficiency in Iron Deficiency

...Until the investigations were complete the patients were given a diet relatively low in folate content, folate-rich foods such as liver and kidney being excluded.

The group consisted of 38 females and 12 males, aged between 24 and 87 yr, with a mean age of 53.8 yr. The patients were studied before their anaemia was treated and again 6 weeks after correction of the iron deficiency with a single total-dose infusion of iron-dextran (Infumir).

**Laboratory Methods:**

**Hematological studies** were carried out using standard methods (Dacie & Lewis, 1968). All the hematological observations in this study were made by one observer (P.D.R.). Bone marrow films were stained both by May–Grünwald-Giemsa and by Perl’s method for iron. Marrow fragments and 100 consecutive normoblasts were examined for stainable iron. Fragments of normal marrow always show stainable iron, with one to four stainable iron granules in 39–52% of the normoblasts (Bainton & Finch, 1964).

**Normochromic normoblast counts** were performed on stained peripheral blood films; 100 normoblasts were examined in each film and the percentages of cells with five lobes and with more than five lobes, and the mean nuclear-lobe counts were recorded (Herbert, 1959). In a normal series of 50 non-anaemic healthy subjects taken at random from hospital staff and counted by the same observer the mean lobe counts ranged from 2.59 to 3.36, with a mean of 3.09. No control subjects showed cells with more than five lobes but 14 (28%) had more than 10% of five-lobed cells. These counts were also compared with those on 50 non-anaemic hospital out-patients.

**Serum-folate levels** were measured by microbiological assay with Lactobacillus casei A.T.C.C. 1079هذه (L. casei), using the method of Waters & Mollin (1961); our unequivocal normal range by this method is 6–21 ng/ml. Patients with megaloblastic anaemia due to folate deficiency usually have levels below 3 ng/ml. Red-cell-folate levels were measured by estimating the L. casei activity of haemolysates of sequestered whole blood samples (Hoffbrand et al., 1966). Our normal range is 160–640 ng/ml packed red cells. This estimation was performed on 37 unselected patients in the series of 50 before iron therapy and in 26 following therapy.

**Serum-vitamin-B_{12} levels** were assayed by the method of Andersson (1964), using the 'z' strain of Euglena gracilis. With this method our normal range is 160–925 pg/ml.

**Figh and uracil acid** were measured in 47 patients in an 8-hr urine specimen collected after an oral dose of 13 g u-histidine monohydrochloride, by the spectrophotometric method of Linnarzin & Bennett (1962), which measures both Figh and uracil acid. Subjects with defective folate metabolism may excrete more than 17 mg of Figh in the 8-hr period. When the amount of the two compounds was 17 mg or less the result was recorded as Figh; with results over 17 mg uracil acid was estimated separately and the amount subtracted from the total.

**RESULTS**

**Findings before Iron Therapy**

**Haemoglobin range.** The haemoglobin range for this group of patients was 3.1–10.3 g/100 ml, with a mean of 7.7 g/100 ml.
globin levels lay anywhere within the range for this group. However, the nine patients with haemoglobin levels above 9.5 g/100 ml did not show giant metamyelocytes and all five patients with haemoglobin levels below 5.5 g/100 ml showed both multilobing and hyper-segmented cells.

There was no correlation between the degree of anaemia and the serum-folate levels, red-cell folate levels or Figlu excretion. In particular, the four patients with subnormal red-cell folate levels were evenly distributed through the haemoglobin range.

<table>
<thead>
<tr>
<th>Serum folate (ng/mL)</th>
<th>Red cell folate (ng/mL packed cells)</th>
<th>Urine Figlu (mg/Bir)</th>
<th>Serum B_{12} (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 50</td>
<td>27</td>
<td>47</td>
<td>60</td>
</tr>
<tr>
<td>Range 11–18–4</td>
<td>94–968</td>
<td>0–437</td>
<td>80–960</td>
</tr>
<tr>
<td>Mean 5.7</td>
<td>374</td>
<td>39</td>
<td>347</td>
</tr>
</tbody>
</table>

![Graph](image)

**Fig. 1.** Serum and red-cell folate levels, urine Figlu excretion and serum vitamin-B_{12} levels in patients with hypochromic anaemia. Dashed lines indicate normal ranges (see "Laboratory Methods").

**Serum-folate levels.** There was no correlation between morphological changes in the neutrophil series and serum-folate levels. Two of the patients with serum-folate levels of less than 3.0 ng/ml showed neither abnormal neutrophil lobing nor marrow giant metamyelocytes.

The serum-folate levels were less than 6.0 ng/ml in all four patients with red-cell folate
levels below 160 ng/ml, and in three of these patients serum-folate levels were within 0–2.9 ng/ml. Two patients with serum-folate levels below 3.0 ng/ml had normal red-cell folate levels.

The mean serum-folate level for patients with a negative Figlu test was 6.4 ng/ml, compared with a mean level of 5.0 ng/ml for those with a positive Figlu test. There was, however, no correlation in individual cases between the Figlu test and serum-folate level; eight patients with serum-folate levels of more than 6.0 ng/ml had positive Figlu tests, and five patients with serum-folate levels of less than 3 ng/ml had negative Figlu tests.

Red-cell folate levels. All four patients with red-cell folate levels below 160 ng/ml of packed red cells showed neutrophil multilobing; one of these had hypersegmented cells and two had giant metamyelocytes in the marrow. The mean red-cell folate was 612 ng/ml of packed red cells in patients showing no neutrophil multilobing, compared with a mean of 355 ng/ml for patients showing these abnormalities. Patients with no giant metamyelocytes in the marrow had a mean red cell folate of 433 ng/ml compared with a mean level of 299 ng/ml for those showing these abnormal cells.

Figlu excretion. As already described, the incidence of abnormal Figlu excretion did not correlate with the degree of anaemia and there was little correlation with the serum-folate levels. There was no correlation between abnormal Figlu excretion and the incidence of abnormal red-cell folate levels or the presence of abnormal neutrophil lobing and giant metamyelocytes.

Correlation with the clinical state. Twenty-six patients were below the age of 60 and 24 were 60 and over. There was no significant difference in the findings between these two age groups. There were also no significant differences related to the clinical diagnoses.

Table II. Haemoglobin rise 6 weeks after iron infusion, related to the initial serum folate level

<table>
<thead>
<tr>
<th>Serum folate (ng/ml)</th>
<th>No. of patients</th>
<th>Hb rise (g/100 ml)</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2.9</td>
<td>12</td>
<td>0.7–5.7</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>3–5.9</td>
<td>18</td>
<td>1.4–7.2</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>6+</td>
<td>20</td>
<td>1.4–8.6</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>

Results Following Iron Therapy

The 50 patients were restudied 6 weeks after correction of the iron deficiency by a single total-dose intravenous infusion of iron-dextran. The dose of iron-dextran was calculated from body weight and haemoglobin level according to the manufacturer’s instructions (Total iron given (mg) = 0.3 x Body weight x Haemoglobin deficit in percentage. 1 ml ‘Imferon’ = 50 mg iron).

Haemoglobin changes. Six weeks after iron infusion the haemoglobin range was 9.8–14.7 g/100 ml, with a mean of 12.2 g/100 ml. The haemoglobin increase ranged from 0.7 to 8.6 g/100 ml with a mean rise of 4.5 g/100 ml, and showed the expected inverse relationship to the pre-treatment haemoglobin level.
The mean rise in haemoglobin was related to the initial serum-folate level (Table I). The patients were divided into three groups, with serum folate levels of 0-2.9, 3-5.9 and 6.0-9.9 ng/ml. The range of haemoglobin responses in each group was wide but comparing the three groups, the differences between the means of the increase in haemoglobin were significant ($P<0.01$). The results were closely similar whether based on the pre- or post-treatment serum-folate levels. There was no correlation between haemoglobin rise and changes in the serum-folate levels over the 6 weeks' post-treatment period. However, the mean haemoglobin rise was higher, 5.0 g/100 ml, in the 22 patients with normal red-cell folate levels, compared with a mean rise of 3.6 g/100 ml in the four patients with subnormal red-cell folate levels: the number of patients with subnormal red-cell folate levels was too small to determine a statistically significant difference.

The mean haemoglobin rise was 4.8 g/100 ml in patients with negative Hist-Gates and 4.0 g/100 ml in those with positive Figgio tests, but the difference was not statistically significant. There was no significant difference in haemoglobin response between patients who did or did not show neutrophil multilobing, hypersegmented cells or giant metamyelocytes.

In each of the five patients with serum vitamin-B₁₂ levels below 166 pg/ml before iron therapy the haemoglobin rise with treatment was within the range of increase for their particular serum-folate group, three being in the 3.0-5.9 ng/ml group and two having serum folate levels below 3.0 ng/ml. The mean haemoglobin increase for these five patients was 8.9 g/100 ml compared with the overall mean increase of 4.5 g/100 ml.

**Table III. Changes in neutrophil multilobing and marrow giant metamyelocytes 6 weeks after iron infusion.**

<table>
<thead>
<tr>
<th></th>
<th>Pre-infusion</th>
<th>Post-infusion</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range of mean lobe counts</td>
<td>2.8-4.2</td>
<td>2.0-4.5</td>
<td></td>
</tr>
<tr>
<td>Mean of mean lobe counts</td>
<td>3.47</td>
<td>3.27</td>
<td></td>
</tr>
<tr>
<td>Hypersegmented cells</td>
<td>40%</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>Giant metamyelocytes</td>
<td>38%</td>
<td>22%</td>
<td></td>
</tr>
</tbody>
</table>

**Changes in the parameters suggesting folate deficiency.** The morphological changes suggesting folate deficiency in the presence of a normal serum vitamin-B₁₂ level, i.e., multilobe mean lobe count with hypersegmented cells in the peripheral blood and giant metamyelocytes in the bone marrow, showed significant changes 6 weeks after iron infusion (Table III). Although the range of the mean lobe count was wider than before iron therapy, the mean of this the range of the mean lobe count was wider than before iron therapy, the mean of this range was not significantly higher. The number of mean lobe counts greater than 3.30 fell from 35 (70%) before therapy to 24 (48%) after therapy. In 39 patients the mean lobe counts fell, moving from abnormal into the normal range; in 20 patients the mean lobe counts fell, moving from abnormal into the normal range; in 20 patients the mean lobe counts fell, moving from abnormal into the normal range; in 20 patients the mean lobe counts fell, moving from abnormal into the normal range; in 20 patients the mean lobe counts fell, moving from abnormal into the normal range; in 20 patients the mean lobe counts fell, moving from abnormal into the normal range. Similarly there was a highly significant fall in the proportion of patients with giant metamyelocytes in the bone marrow. The bone marrow did not precipitate a megaloblastic change in any of the marrows.
The mean serum- and red-cell folate levels showed a highly significant fall 6 weeks after iron infusion (Table IV). In 34 patients (68%) the serum-folate levels fell, with a rise in 14 (28%) and no change in two patients (4%). The red-cell folate levels fell in 22 patients (85%) of those tested and rose in four (15%).

The amount of Figlu excreted after a histidine load did not show any significant pattern of changes following iron therapy. The amount excreted rose in 20 patients (43% of those tested) and fell in 27 patients (57%). Twenty-two patients (47%) excreted an abnormal amount of Figlu after treatment compared with 21 (45%) before iron therapy.

**Table IV. Changes in serum and red-cell folate levels 6 weeks after iron infusion**

<table>
<thead>
<tr>
<th></th>
<th>Pre-infusion</th>
<th>Post-infusion</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.1–18.4</td>
<td>0.9–9.4</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>5.7</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Red cell folate (ng/ml packed cells)</td>
<td>94–968</td>
<td>61–424</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>374</td>
<td>225</td>
<td></td>
</tr>
</tbody>
</table>

**Changes in serum-vitamin-B$_{12}$ levels.** In patients with normal serum-vitamin-B$_{12}$ levels both before and after iron infusion, the levels fell in 22 (44%) and rose in 22 (44%). In four of the patients with subnormal levels before iron therapy, the levels were still in the subnormal range following therapy. In one patient the serum-vitamin-B$_{12}$ level rose from a subnormal figure of 150 pg/ml to within the normal range at 265 pg/ml. One low normal pre-treatment level of 180 pg/ml fell to a subnormal level of 150 pg/ml.

Correlation of data suggesting folate deficiency. Following the iron therapy there was even less correlation between the various tests for folate deficiency than before treatment.

**DISCUSSION**

The most striking feature to emerge from this study of patients with untreated simple hypochromic anaemia was a high incidence of morphological changes in the neutrophil series. Many of these patients showed high mean lobe counts, hypersegmented neutrophils and giant metamyelocytes in the bone marrow. Abnormal Figlu tests were common and in many patients the serum-folate levels were low. In contrast the mean red-cell folate level was higher than the normal range for the method used, an observation which agreed with the results of Hansen (1964), who found high whole-blood folate levels in iron-deficiency anaemia, with a much greater range than in the normal. These high red-cell folate levels could be related to an increased population of young red cells in the circulation (Hoffbrand et al., 1966) but in the present series they were not related to the reticulocyte counts. It is probable that the red-cell folate level, as a measure of tissue folate, is the best indicator of significant folate deficiency and the feature least disturbed by coexisting iron deficiency.

If the morphological changes in the neutrophil series, low serum-folate levels and positive Figlu tests were indications of significant folate deficiency in these iron-deficient patients, the...
Folate Deficiency in Iron Deficiency

Deficiency might have been due to an additional demand for folate or an abnormality of folate metabolism produced by the iron deficiency (Chanarin et al., 1965), possibly in turn due to an increase in the amount of ineffective erythropoiesis; in this context the high red-cell folate levels are of particular interest.

The additional demand for folate may precipitate changes of folate deficiency in subjects with combined subclinical folate and iron deficiency. The abnormal Figlu excretion may be a reflection of this folate deficiency or a direct result of the effect of iron deficiency on liver formiminotransferase (Vitale et al., 1965a, b, 1966), although the work of Burns & Spray (1969) does not support this hypothesis.

The changes following the correction of iron deficiency in these patients suggests that the response to iron therapy is modified by coexisting folate deficiency as assessed by the serum- and red-cell folate levels, but not as assessed by the presence of giant metamyelocytes in the narrow, multilobing of the peripheral blood neutrophils or a positive Figlu test. Although the iron therapy produced a fall in both serum- and red-cell-folate levels, the morphological changes suggesting folate deficiency became less marked, with no significant change in the results of the Figlu test. In no patient did the marrow become megaloblastic following iron treatment.

In iron-deficiency anaemia there has been little investigation of the effect of folate status on the response to iron therapy, and of the effect of iron therapy on folate status. Velez et al. (1966) noted a fall in serum-folate levels following iron therapy in four out of six patients with iron deficiency. Hines et al. (1967) restored normal haemoglobin levels in a series of post-gastrectomy patients with evidence of iron and folate deficiency, using iron therapy alone; they noted no apparent alterations in the folate status of these patients. Hoffbrand et al. (1967) compared the serum-folate levels of a few post-gastrectomy patients before and after iron therapy and noted no significant change, but they did not record the rate of response to iron therapy and noted no significant change, but they did not record the rate of response to iron therapy. Previous workers have noted a delay in the rise in haemoglobin after oral iron in anaemic post-gastrectomy patients (Baird et al., 1959). In assessing post-partum patients 6 weeks after intravenous iron infusion, Metz et al. (1967) noted a greater rise in haemoglobin when folic acid was given in addition to the iron, and a deterioration in folate status when iron was given alone, compared with similar patients given no iron therapy. Parenteral iron therapy in anaemic ante-natal patients may lead to signs of folate deficiency (Combrink et al., 1966) and these complications are avoided by the administration of folic acid with the iron therapy (Varde, 1964; Goldshoer et al., 1965). It is probable that the response to iron therapy increases the demand for folate and that the falls in both serum- and red-cell-folate levels observed are an indication of the depletion of folate stores. This suggestion would explain why the haemoglobin rose more rapidly in those of our patients who had normal serum-folate levels. Furthermore, although liver and kidney were excluded from patients’ diets throughout the study, it is possible that patients ate more folate after iron infusion because of increased appetite. This would make the fall in serum and red-cell folate all the more remarkable.

The observation that marrow giant metamyelocytes and multilobing of the peripheral blood neutrophils became less marked after iron therapy, despite falls in serum- and red-cell-folate levels, suggests that these cell changes may, under certain conditions, be related at least to iron deficiency. The occurrence of these cells in iron-loaded classical megaloblastic anaemia shows that their relationship to iron deficiency is not simple. It is possible that in
iron deficiency folate metabolism is not normal. Chanarin et al (1965) noted the highest incidence of multilobed polymorphs in ante-natal patients who were iron deficient. Hansen (1964) observed that marrow giant metamyelocytes showed no change after 3 days of iron therapy but after 3 weeks of therapy these cells had disappeared. In post-partum anaemia neutrophil multilobing and giant metamyelocytes were less evident 6 weeks after intravenous iron-infusion (Metz et al, 1967), and Beard & Weintraub (1969) have also noted neutrophil multilobing in iron deficiency which disappeared with oral iron therapy.

The high incidence of abnormal Figlu excretion similarly suggests that this may be related in some way to iron deficiency. There is some evidence that liver formiminotransferase may be iron dependent (see above), and the highest Figlu excretions in pregnancy are found in iron-deficient subjects (Chanarin et al, 1962, 1965). The presence of a positive Figlu test in the present series did not affect the response to iron therapy, nor did the iron therapy have any significant effect on Figlu excretion, as measured 6 weeks after iron infusion.

It was anticipated that the data suggesting folate deficiency would be less well correlated in the presence of iron deficiency. However, 6 weeks after iron replenishment the correlation was even less significant. It is possible that at 6 weeks these patients were still responding to the iron therapy and that a significant correlation of folate-deficiency data might only emerge when they were haematologically stable. In this respect our findings are at variance with those of other centres. We suggest that observer differences on neutrophil lobe counts and difficulties in comparing the results of folate assays between laboratories may have contributed to this discrepancy.

The slightly low serum-vitamin-B₁₂ levels found in five patients (four with partial gastrectomy) did not significantly influence the response to iron therapy. The response in these few patients is in contrast to the observations of Hines et al (1967), in whose post-gastrectomy series only a few iron- and vitamin-B₁₂-deficient patients showed a return of haemoglobin to normal, and these few then showed macrocytosis and multilobed neutrophils. The vitamin-B₁₂ deficiency in these patients was probably more severe than in the present series, where no macrocytosis or marrow megaloblastic changes appeared after iron therapy. Although some investigators have found a significant rise in serum-vitamin-B₁₂ levels on treating iron-deficient post-gastrectomy patients with iron (Cox et al, 1959; Jones et al, 1962; Williams, 1964), the vitamin-B₁₂ levels showed no significant changes in our series. This is in accord with the results in most of the post-gastrectomy patients reported by Hoffbrand et al (1967), although three out of 39 of their subjects showed a significant rise in serum-vitamin-B₁₂ levels. In post-partum anaemia Metz et al (1967) found no significant change in serum-vitamin-B₁₂ levels following intravenous iron infusion.

It has been suggested that major reactions to intravenous iron therapy occur in patients with folate deficiency (Lane & Scott, 1965). Metz et al (1967) noted no such significant association and in the present series, although evidence of folate deficiency was common, no major reactions were encountered.

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Folate Deficiency in Iron Deficiency

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