Iron Deficiency and Dyserythropoiesis

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SUMMARY. In 10 patients with Fe deficiency significant dyserythropoiesis was found. The degree of dyserythropoiesis provided a reliable indication of the severity of the iron deficiency as measured by the serum iron and total iron binding capacity; a positive correlation \( P < 0.002 \) being found between the incidence of dyserythropoiesis in the bone marrow and both the serum iron and \% iron saturation. The frequent occurrence of basophilic stippling in the erythroblasts of these patients suggested that the presence or absence of stippling does not help differentiate between iron deficiency and other causes of hypochromic anaemia.

The diagnostic features of iron deficiency have been described in several recent reviews (Undritz, 1964; Bainton & Finch, 1964; Wintrobe, 1967; Charlton & Bothwell, 1970). In the bone marrow there is usually erythroid hyperplasia with a predominance of intermediate normoblasts. The normoblasts have abnormal morphology, many showing reduced ragged cytoplasm, sometimes with vacuolation and delayed cytoplasmic maturation. However, the nuclear changes of karyorrhexis, erythroblast multinuclearity, abnormal mitosis, nuclear budding and fragmentation, or the occurrence of cytoplasmic basophilic stippling have not generally been regarded as typical features of iron deficiency. These appearances are seen characteristically in patients with congenital dyserythropoietic anaemia (Heimpel & Wendt, 1968; Crookston et al., 1969; Verwilghen et al., 1969; Wolff & von Hofe, 1951), and may occasionally occur in a number of other blood diseases including myelosclerosis, leukaemia, aplastic anaemia and in kwashiorkor or following ionizing radiation (Lewis, 1969). Profound biochemical disturbances have recently been found in the erythroblasts of patients with chronic iron deficiency; these include substantial decreases in DNA synthetic rate and cellular nucleic acid content, and abnormal incorporation of labelled iron and glycine into haem (Hershko et al., 1970). The normally balanced synthesis of the \( \alpha \) and \( \beta \) globin chains in erythroblasts has also been shown to be altered in iron deficiency (J. M. White, personal communication, 1971). The possibility that these biochemical abnormalities may result in marked morphological changes led us to re-examine the bone marrow morphology from proven, uncomplicated cases of iron deficiency for evidence of dyserythropoiesis. In an effort to relate the severity of dyserythropoiesis to the degree of iron depletion, morphological features were correlated with biochemical data.

MATERIALS AND METHODS

Bone marrow records over the previous three years were systematically examined, amongst

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these being 215 consecutive entries with the diagnosis of iron deficiency, in patients admitted to this hospital. All but 67 of these were excluded on the basis of a complicating disease, clearly documented, or other bone marrow changes.

Ten cases of uncomplicated iron deficiency were separated from the remaining 67 persons. Patients were included in this series only after the diagnosis had been established on the basis of all of the following criteria: a hypochromic microcytic peripheral blood film, reduced serum iron, reduced serum iron saturation, absence of stainable iron in bone marrow fragments and normoblastic or micronormoblastic erythropoiesis in the bone marrow. No patient was included unless serum vitamin B₁₂ was > 190 pg/ml, serum folate was > 3 ng/ml, and the blood urea was within the normal range (20–45 mg/100 ml). Patients found to have primary diseases either not directly contributing to the iron deficiency, or known to be a cause of dyserythropoiesis per se (Table I) were excluded. All the patients studied presented with a clinical history compatible with a diagnosis of iron deficiency, and none had undergone splenectomy. Blood films and bone marrow specimens were prepared and stained by standard methods (Dacie & Lewis, 1968). Serum vitamin B₁₂ and folate levels were measured by microbiological assays and serum iron, iron binding capacity and blood urea were estimated using established techniques (Dacie & Lewis, 1968; Skeggs, 1957).

The percentage of dyserythropoietic and basophilic stippled cells was obtained by the following method: slides for examination were randomized and relabelled to eliminate patient identification. Individual slides were counted by three of the authors, each person counting 200 cells, and then passing on the slide until a minimum of 1000 cells were scanned (five separate counts of 200 cells from three persons). Data was processed and statistical evaluation carried out in conjunction with the Department of Statistics. An erythroblast was identified as dyserythropoietic if one or more of the following nuclear morphological features were present: karyorrhexis, nuclear budding or fragmentation, multinuclearity and nuclear bridging. These nuclear criteria for dyserythropoiesis have been adopted from recent reviews (Lewis, 1969). Because iron deficiency is associated with considerable cytoplasmic abnormality, cytoplasmic changes including basophilic stippling and vacuolation, and cytoplasmic inclusions such as Howell-Jolly bodies were not included within our definition of dyserythropoiesis and were considered separately. Bone marrow neutrophil lobe counts were performed on stained marrow films. Not less than 200 neutrophils were counted in each slide. Ten morphologically normal bone marrows served as controls.

RESULTS

The relevant clinical, biochemical and morphological findings are summarized in Table I. The morphological appearances of two of the patients with widely differing dyserythropoietic counts (patients 2 and 10) are illustrated in Fig 1. Marked karyorrhexis, nuclear budding and fragmentation were invariable findings. Erythroblast multinuclearity was also found but occurred less frequently. Punctate basophilia was found in all cases and was generally, although not always, most prominent in the most severely dyserythropoietic bone marrows (Table I). Karyorrhexis was, however, the only consistently prominent feature in mild iron deficiency.

Case 10, a patient whose iron depletion had been caused by self-bleeding and whose history, clinical findings and investigations have been reported in detail elsewhere (Tattersall et al,
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Fig 1. Photomicrographs of bone marrows showing dyserythropoiesis from: (A) case 2, <10% abnormal erythroblasts; (B) case 10, 46% abnormal erythroblasts. (x 600).
TABLE I. Clinical, biochemical and morphological data from 10 patients with iron deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex and age (yr)</th>
<th>Diagnosis</th>
<th>Hb (g/100 ml)</th>
<th>Urea (mg/100 ml)</th>
<th>Serum Fe (µg/100 ml)</th>
<th>TIBC(Fe) (µg/100 ml)</th>
<th>%Fe satn</th>
<th>Serum vitamin B₁₂ (pg/ml)</th>
<th>Serum folate (ng/ml)</th>
<th>Punctate basophilia (% of erythroblast ct) ± 1 SD</th>
<th>Dyserthropoiesis (%) ± 1 SD†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F 83</td>
<td>? Dietary deficiency</td>
<td>9.1</td>
<td>40</td>
<td>49</td>
<td>430</td>
<td>11.4</td>
<td>280</td>
<td>6.4</td>
<td>3.0 ± 1.7</td>
<td>5.1 ± 2.2</td>
</tr>
<tr>
<td>2</td>
<td>F 34</td>
<td>Menorrhagia</td>
<td>10.1</td>
<td>25</td>
<td>60</td>
<td>405</td>
<td>18.8</td>
<td>224</td>
<td>10.4</td>
<td>0.7 ± 0.6</td>
<td>6.5 ± 3.2</td>
</tr>
<tr>
<td>3</td>
<td>F 54</td>
<td>? Dietary deficiency</td>
<td>5.8</td>
<td>26</td>
<td>30</td>
<td>408</td>
<td>7.3</td>
<td>408</td>
<td>8.2</td>
<td>1.4 ± 0.4</td>
<td>12.7 ± 2.6</td>
</tr>
<tr>
<td>4</td>
<td>M 33</td>
<td>Haemorrhoids</td>
<td>6.6</td>
<td>24</td>
<td>20</td>
<td>410</td>
<td>4.9</td>
<td>275</td>
<td>4.4</td>
<td>2.1 ± 0.7</td>
<td>13.5 ± 2.7</td>
</tr>
<tr>
<td>5</td>
<td>F 32</td>
<td>Postpartum</td>
<td>10.8</td>
<td>20</td>
<td>45</td>
<td>410</td>
<td>10.9</td>
<td>496</td>
<td>162*</td>
<td>2.3 ± 0.3</td>
<td>15.2 ± 4.3</td>
</tr>
<tr>
<td>6</td>
<td>F 31</td>
<td>Hookworm infestation</td>
<td>5.4</td>
<td>15</td>
<td>30</td>
<td>420</td>
<td>7.1</td>
<td>520</td>
<td>4.6</td>
<td>1.7 ± 0.6</td>
<td>20.4 ± 6.2</td>
</tr>
<tr>
<td>7</td>
<td>M 58</td>
<td>Peptic ulcer</td>
<td>6.3</td>
<td>27</td>
<td>5</td>
<td>450</td>
<td>1.1</td>
<td>312</td>
<td>3.2</td>
<td>3.5 ± 1.6</td>
<td>25.6 ± 4.4</td>
</tr>
<tr>
<td>8</td>
<td>F 55</td>
<td>? Dietary deficiency</td>
<td>5.7</td>
<td>40</td>
<td>20</td>
<td>528</td>
<td>3.8</td>
<td>408</td>
<td>9.8</td>
<td>2.6 ± 1.8</td>
<td>27.2 ± 8.5</td>
</tr>
<tr>
<td>9</td>
<td>M 48</td>
<td>Peptic ulcer, GI bleeding</td>
<td>4.2</td>
<td>24</td>
<td>19</td>
<td>420</td>
<td>4.5</td>
<td>196</td>
<td>13.4</td>
<td>8.7 ± 3.8</td>
<td>28.6 ± 4.2</td>
</tr>
<tr>
<td>10</td>
<td>M 29</td>
<td>Self-bleeding</td>
<td>3.9</td>
<td>20</td>
<td>16</td>
<td>432</td>
<td>3.7</td>
<td>256</td>
<td>8.6</td>
<td>3.8 ± 1.5</td>
<td>46.2 ± 7.0</td>
</tr>
</tbody>
</table>

* Red cell folate (normal range 160–640 ng/ml).
† See text.
1972), showed a number of remarkable morphological features (Fig 1B and 2). At least 46% of his erythroblasts showed striking dyserythropoietic changes, most prominent being karyorrhexis, nuclear fragmentation and budding. Occasional erythroblast internuclear bridges were found. This is of particular interest in view of the fact that its occurrence has been well recognized in primary (congenital) dyserythropoietic anaemia (Heimpel & Wendt, 1968). Although punctate basophilia was not particularly impressive, none the less basophilic stippling was present in 3.8% of the total nucleated red cells, and the presence of coarsely stippled cells was a striking feature of the marrow morphology.

![Graph showing the relationship of dyserythropoiesis to serum Fe.](image)

**Fig. 3.** The relationship of dyserythropoiesis to serum Fe.

The serum iron and iron saturation estimations plotted against the dyserythropoietic counts in the bone marrows are shown in Figs 3 and 4. Serum iron and iron saturation values, but not iron binding capacity, were positively related to the degree and severity of dyserythropoiesis. The correlation in each instance was highly significant ($P<0.002$), and in this small series the percentage of dyserythropoiesis provided a reliable indication of the degree of iron depletion. Because of the unequivocal presence of giant granulocytes in at least five of the ten bone marrows, lobe counts of marrow neutrophils were performed. There was no difference in the mean lobe counts between the iron deficient patients (mean of $2.01 \pm 0.27$) and 10 morphologically normal marrows (mean of $2.02 \pm 0.13$).

**DISCUSSION**

A group of iron deficient patients are described in whom dyserythropoiesis of varying degrees was noted. The most marked morphological abnormalities were usually seen in those patients most profoundly iron-depleted. The most striking and constant morphological feature noted was karyorrhexis. Nuclear budding, multinuclearity and fragmentation were, however, also present in varying degrees. In addition, in at least one case, internuclear bridging was observed. This latter finding is of particular interest in that it has previously been considered a particular feature of one type of congenital dyserythropoietic anaemia
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(Heimpel & Wendt, 1968); indeed this patient was initially considered as suffering from this disorder because of this finding.

The finding of giant granulocytes is also of interest; the presence of giant granulocytes in iron deficiency has been a matter of controversy. Several recent reports, however, have drawn attention to their presence in iron deficiency (Beard & Weintraub, 1969), and the morphology has been noted to revert towards normal after the institution of iron therapy. Recent work also suggests a relationship between iron deficiency and defective folate metabolism (Chanarin et al, 1962, 1965; Cusack & Brown 1964; Vitale et al, 1966), although the essential connection between them is not known. In our present group of patients, giant granulocytes occurred in iron-deficient marrows, irrespective of the folate levels. Furthermore, we could find no increase in the mean lobe counts of marrow neutrophils from iron-deficient patients compared to normal controls, nor could we relate Howell-Jolly bodies to serum folate levels, to suggest that other parameters of possible folate ‘deficiency’ were present. We have attributed the presence of dyserythropoiesis to the direct effect of iron deficiency. Dyserythropoiesis is a well-recognized association of megaloblastic erythropoiesis. However, cases 8, 9 and 10, the patients showing the most striking changes, had high serum folate levels, normal levels of vitamin B₁₂ and no morphological features of megaloblastic erythropoiesis. Our results also support the view that significant basophilic stippling can occur as a direct consequence of iron deficiency. The presence of basophilic stippling is at variance with the teaching that a distinction can be made on the basis of the presence or absence of punctate basophilia between severe iron deficiency and other causes of hypochromic anaemia such as thalassaemia, sideroblastic anaemia and lead poisoning. The pathogenesis of the abnormalities described here in iron deficiency is not yet known, but these observations underline the probability that iron has an important role in erythroblast DNA and conceivably RNA synthesis and it has been suggested that it is necessary for the preservation of orderly nuclear maturation in erythropoiesis (Hershko et al, 1970; Cusack & Brown, 1964; Vitale et al, 1966).
ACKNOWLEDGMENTS

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REFERENCES


