CASE REPORT

Erythroblastic Anemia
A Manifestation of Folic Acid Deficiency

By H. O. Nieweg and A. Arends, M.D.

IN CONJUNCTION with the sprue syndrome one frequently observes anemia. This anemia is often hypochromic, but may be hyperchromic if an iron deficiency is predominant. Less common is the erythroblastic anemia of sprue first described by Bennet, Vaughan and Hunter in 1932. It is characterized by the occurrence of normoblasts in the peripheral blood. As van Buchem pointed out, these normoblasts are of the orthochromatic type and have pyknotic nuclei, which allows one to differentiate this form of anemia from that accompanied by extramedullary hematopoiesis, where the normoblasts in the peripheral blood are polychromatophilic or basophilic. Bennet, Vaughan and Hunter do not mention autopsy findings, but Hirschfeld and Dummer observed atrophy of the spleen. This association of splenic atrophy and erythroblastic anemia was also found by subsequent authors. After splenectomy one may temporarily observe similar changes in the peripheral blood.

We observed 5 cases of erythroblastic anemia in which postmortem examination showed the existence of splenic atrophy. No foci of extramedullary hematopoiesis were found in the liver in these cases. Steatorrhea was observed in 4 of the 5 cases. Case 4 died of chronic glomerulonephritis, after therapy with a low protein diet for one year. More details will be given about Case 5 as it offers an opportunity to discuss the etiology of this syndrome. Data on all 5 cases are summarized in table 1.

DISCUSSION

The cause of the occurrence of Howell-Jolly bodies and of orthochromatic normoblasts after splenectomy has been the subject of much discussion. Van Buchem believes that the normal spleen and reticulo-endothelial system exert a humoral influence on the denucleation of red cells in the bone marrow.

Another point of discussion is the cause of the atrophy of the spleen in certain cases of sprue. De Vries thought it might be due to a deficiency, without stating which specific factor was lacking. Our observation (Case 4) on the occurrence of splenic atrophy in a young man, who was given an inadequate diet without a sufficient vitamin supplement over a long period for a chronic glomerulonephritis, favors this hypothesis.

The animal experiments performed by Asenjo throw a new light on the possible

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### Table 1—Summary of the Findings in 5 Cases of Erythroblastic Anemia

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Duration of illness</th>
<th>Total fat content in % of the dry weight of the feces (normal up to 30%)</th>
<th>Peripheral blood</th>
<th>Bone marrow</th>
<th>Autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. m.</td>
<td>58</td>
<td>3 years</td>
<td>37-54%</td>
<td>Macrocystic, Howell-Jolly bodies</td>
<td>Megaloblasts</td>
<td>Spleen 10 Gm.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Orthochromic erythroblasts</td>
<td>Giant stabs</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Leukopenia</td>
<td>Refractory to refined liver</td>
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<tr>
<td>2. f.</td>
<td>47</td>
<td>1½ years</td>
<td>37%</td>
<td>Macrocystic, Leukopenia</td>
<td>Hypoplastic</td>
<td>Spleen 14 Gm.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Many Howell-Jolly bodies</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Thrombopenia</td>
<td></td>
<td></td>
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<tr>
<td>3. f.</td>
<td>57</td>
<td>6 years</td>
<td>60%</td>
<td>Macrocystic anemia</td>
<td>Hypoplastic</td>
<td>Spleen 15 Gm.</td>
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<td></td>
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<td></td>
<td>Howell-Jolly bodies</td>
<td></td>
<td>Bronchitis</td>
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<td>Orthochromic erythroblasts</td>
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<td>Pyelonephritis</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Leukopenia</td>
<td></td>
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<tr>
<td>4. m.</td>
<td>18</td>
<td>1 year treated for 1 year with low protein diet</td>
<td></td>
<td>Orthochromic erythroblasts</td>
<td></td>
<td>Spleen 10 gm.</td>
<td></td>
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<td></td>
<td></td>
<td>Chronic glomerulonephritis</td>
<td></td>
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<tr>
<td>5. m.</td>
<td>36</td>
<td>1 year</td>
<td>51%</td>
<td>Macrocystic anemia</td>
<td>Hyperplastic</td>
<td>Spleen 12 Gm.</td>
<td></td>
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<td></td>
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<td></td>
<td>Leukopenia</td>
<td>Megaloblasts and giant stabs</td>
<td>See autopsy findings p. 177</td>
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<td></td>
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<td></td>
<td>Orthochromic erythroblasts</td>
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<td></td>
<td></td>
<td></td>
<td>Howell-Jolly bodies</td>
<td>Increase of orthochromatic nucleated cells</td>
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correlation between splenic atrophy and deficiency. After keeping rats on a folic acid deficient diet, leukopenia, diarrhea, loss of hair and lesions of the oral mucosa developed. In addition, however, atrophy of the spleen was seen, sometimes accompanied by infarction. When Asenjo added a small but inadequate amount of folic acid to the diet, the animals remained alive for a longer period and atrophy of the spleen was observed more frequently. The following report shows the effect of folic acid in one of our cases (Case 5).

Case Report

A 36 year old man had been treated for chronic fibrotic-bronchiecatic pulmonary tuberculosis for about a year. His general condition declined during this period, yet could not be explained on the basis of the pulmonary findings. He was therefore referred to us for a complete examination.

The patient complained of anorexia and loss of weight; physical examination revealed only a poor state of nutrition and anemia. Laboratory data follows:

- Hgb. 62 per cent; RBC 3,230,000; WBC 4300; reticulocyte count 50 thousand; thrombocyte count (van Herwerden's method) 600,000. Differential count: eosinophils 4, stab 10, polymorphonuclear leukocytes 40, lymphocytes 22, monocytes 24; 14 orthochromatic normoblasts per 100 white cells and numerous Howell-Jolly bodies were seen. Serum iron 129 gamma per cent. Bilirubin, indirect 1 mg. per cent. Bone marrow examination showed an increase of orthochromatic nucleated cells and the occurrence of megaloblasts and giant metamyelocytes.

Further studies showed the existence of an absorption defect. The vitamin A curve after 600,000 International Units showed the following: fasting 4 I.U./10 ml.; after 3 hours 23 I.U./10 ml.; after 6 hours 48 I.U./10 ml.; after 9 hours 19 I.U./10 ml. Examination of feces showed total fat 51 per cent of the dry weight, fatty acids 41 per cent, soaps 37 per cent, neutral fat 22 per cent.

We made the clinical diagnosis of nontropical sprue with an erythroblastic anemia due to splenic atrophy and megaloblastic hematopoiesis.

Treatment consisted of a diet, supported by vitamin B12, 15 gamma per day by intramuscular injection. This had no effect whatsoever and is in agreement with the fact that the vitamin B12 level in the serum before treatment was found to be in the high normal range (0.95 my per ml.). A bone marrow smear made on the fourteenth day showed a persistance of the megaloblastic picture (fig. 1a).

During the vitamin B12 administration the patient developed glossitis and diarrhea. Vitamin B12 was therefore stopped and 30 mg of folic acid was given by mouth. A reticulocyte rise occurred with a maximum of 80 per thousand on the seventh day. The hemoglobin and RBC also increased. The most remarkable finding, however, was the total disappearance of the orthochromatic normoblasts from the peripheral blood. Howell-Jolly bodies became smaller and showed fragmentation (see fig. 1b, c, d; fig. 2).

Slight clinical improvement occurred at first, but the general condition steadily deteriorated; the patient developed generalized hypoproteinemic edema and ascites, and diarrhea became intractable. He died in a state of extreme cachexia six weeks after the start of folic acid therapy which was later supported by large amounts of intravenous albumin.

Autopsy, performed 3 hours after death, revealed an extremely cachectic man, height 1.77 meters, weight 37 Kg. The lungs showed a chronic indurative caseating tuberculosis with two small cavities in the upper lobes. The liver was enlarged, 1765 Gm; cut surface was yellow. It showed a histologic picture of fatty fibrosis. The spleen was very small, 12 G n., 5 x 3 x 2 c.m. (fig. 1e). Histologic examination showed a nearly complete disappear-
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Fig. 1
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ance of the red pulp. Testes were small; microscopical picture showed extreme atrophy of the tubuli (fig. 1g). The mucous membrane of the small intestine showed a loss of the normal mucosal pattern.

The microscopic picture revealed a pronounced atrophy of the epithelium, resulting in disappearance of the villi and numerous small ulcerations (fig. 1f). No Sudan stained fat in the mucous membrane, no foam cells. Mesenteric lymph glands showed a packet of nodules (size about 5 cm. in diameter) on cut surface a yellow material which was partly liquified. Microscopic examination showed a disappearance of the normal lymphoid tissue. The main elements were large foam cells, loaded with fat (Sudan stain positive). These changes in the mesenteric glands are identical with those described in Whipple's disease, however there were no foam cells in the intestinal mucosa. We concluded that this was a case of erythroblastic anemia with steatorrhea and atrophy of the spleen; probably Whipple's disease.

Fig. 2.—Curves showing the effect of administration of vitamin B₁₂, oral folic acid and intramuscular folic acid, on Case 5. Hb = percentage of hemoglobin; R = reticulocytes per thousand; E = orthochromatic erythroblasts per 100 white cells.

This patient had an erythroblastic anemia, a marked atrophy of the spleen being demonstrated at autopsy. A folic acid deficiency was demonstrated by the megaloblastic anemia, refractory to vitamin B₁₂, but reacting to folic acid. Its administration resulted in the disappearance of the erythroblasts in the peripheral blood. As the occurrence of orthochromatic erythroblasts in this case may be considered as a clinical manifestation of atrophy of the spleen, we feel to have

Fig. 1

(a)—Bone marrow picture after fourteen days of treatment with vitamin B₁₂ (15 gamma per day). Note persistence of megaloblastic erythropoiesis.

(b and c)—Peripheral blood before folic acid treatment. There are numerous Howell-Jolly bodies and orthochromatic erythroblasts.

(d)—Peripheral blood after folic acid treatment. The orthochromatic erythroblasts have disappeared and the Howell-Jolly bodies show fragmentation.

(e)—Marked atrophy of the spleen (weight 12 Gm.).

(f)—Microscopic picture of the mucosa of the small intestine. There is pronounced atrophy of the epithelium resulting in the disappearance of the villi.

(g)—Microscopic picture of the testes demonstrating the extreme atrophy of the tubuli.
demonstrated an influence of folic acid on the spleen and perhaps on the entire reticulo-endothelial system. The duration of the treatment may have been too short for the spleen to regenerate as the patient died from liver failure due to the absorption defect.

A few cases of atrophy of the spleen with a megaloblastic bone marrow refractory to liver extract have been described in the literature (Van Hees). As far as we know 1 case of erythroblastic anemia treated with folic acid has been described by Waldenström. He stated that the patient showed subjective improvement, but there was no change in the hemoglobin and red cell count. The serum iron, however, decreased from 60 to 32 gamma per cent.

Folic acid deficiency in man may result in atrophy of the spleen. Sometimes, however, splenomegaly is found in folic acid deficiency, as we know from our experiences with megaloblastic anemia in pregnancy or puerperium. In these cases the disease is usually of short duration and signs of hemolysis may be prominent, which were far less striking in the patients described in this paper. We feel that the time factor may be of great importance for the development of atrophy of the spleen; this was also pointed out by Asenjo.

An explanation for the action of folic acid in maintaining the integrity of the spleen may be found in the current opinion that folic acid is necessary for cytopoiesis. Microbiologic work has further shown that folic acid plays a role in the synthesis of desoxyribose nucleic acids (DNA). That a disordered DNA metabolism is also significant in human pathology is demonstrated by the work of Spies who produced remissions in sprue and pernicious anemia by the administration of thymine, one of the constituents of DNA.

During the process of mitosis the number of chromosomes is doubled involving a twofold increase in the amount of DNA present. It is therefore to be expected that folic acid deficiency may give rise to disturbances in those tissues characterized by a high frequency of cell division.

In tracer studies on the daily turnover of DNA P with P32 it was demonstrated that it is most rapid in the bone marrow and the intestinal tract (15 per cent). This is followed by the spleen (5.8 per cent) and the testes (2.6 per cent).

Another indication of the frequency of cell division in different organs is their sensitivity to radiation damage. As is generally known, irradiation mainly affects the bone marrow, the intestinal tract, the spleen and the testes.

We feel, therefore, that atrophy of the spleen is due to a disturbance of regeneration which may be secondary to a folic acid deficiency. The same mechanism is probably responsible for the atrophy of the testes.

**Summary**

Atrophy of the spleen was observed in 3 cases of nontropical sprue, 1 case of chronic glomerulonephritis and 1 case of Whipple's disease. The occurrence of orthochromatric normoblasts and Howell-Jolly bodies in the peripheral blood in the absence of splenectomy may be a manifestation of atrophy of the spleen. Folic acid therapy was tried in 1 case and resulted in the disappearance of the orthochromatric normoblasts and fragmentation of the Howell-Jolly bodies.

Some arguments are put forward in favor of the conception that atrophy of
the spleen may be caused by a disturbance of cell regeneration due to a chronic folic acid deficiency.

REFERENCES

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