Plasma and Erythrocyte Folate in Iron Deficiency and Folate Deficiency

By A. Omer, N. D. C. Finlayson, D. J. C. Shearmann, R. R. Samson and R. H. Girdwood

It was shown by Toennies et al.¹ that most of the blood folate is within the erythrocytes. The erythrocyte folate level may be reduced in either folate or vitamin B₁₂ deficiency,²⁻⁵ and it can be used as an index of the folate content of body tissues.⁴ The present paper reports that in iron deficiency anemia there are abnormalities in distribution of folate between plasma and erythrocytes which may be corrected by iron treatment alone. The relationships between folate and iron metabolism are discussed.

Subjects and Methods

Subjects

Fifty-seven subjects with normal peripheral blood findings, who were either members of the laboratory staff or blood donors, were used as controls.

Twenty patients with iron deficiency anemia were investigated (hemoglobin 3.8–10.9 Gm./100 ml.). All had a hypochromic blood picture, mean corpuscular hemoglobin concentration 24–30 per cent, a serum iron of less than 30 μg./100 ml. and a total iron binding capacity of greater than 300 μg./100 ml. Marrow was examined in 18 patients and none showed the presence of stainable iron. The reticulocyte counts in the blood samples were less than four per cent. Fourteen of these patients were reinvestigated after treatment with oral iron alone when the hemoglobin had returned to normal. Twenty-three patients with megaloblastic anemia due to folate deficiency were also studied. All had plasma folate values of less than 2.0 ng./ml. and serum vitamin B₁₂ levels above 150 pg./ml.

Methods

General. Routine hematological methods were also described by Dacie and Lewis.⁶ Serum iron was measured by the method of Young and Hicks⁷ and total iron binding capacity by the method of Ramsay.⁸⁻⁹ Serum vitamin B₁₂ was assayed using E. gracilis.¹⁰

Plasma and Erythrocyte Folate. Twenty milliliters of blood from each subject were collected into a heparinized container. The plasma was separated by centrifugation and an aliquot stored at 20°C with ascorbic acid (10 mg./ml. plasma) for plasma folate estimation.

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tion by the method of Herbert\textsuperscript{11} involving the addition of ascorbic acid both to the standard tubes and to those containing the serum, but modified in that Difco Bacto Folic Acid Casei Medium was used. The remainder was recombined with a portion of the erythrocytes and the microhematocrit value determined, after which it was used to estimate erythrocyte folate as follows. Hemolysates were prepared by adding 1 ml. of blood to 9 ml. of freshly prepared one per cent ascorbic acid solution\textsuperscript{4} which produces full folate release within 10 minutes.\textsuperscript{12} The hemolysates were stored at $-20{\degree}C$ until the day of assay when 2 ml. were added to 18 ml. 0.1 M phosphate buffer containing 200 mg. ascorbic acid and the proteins precipitated by autoclaving for five minutes at 15 lbs./sq. in. The extracts were then assayed with \textit{L. casei}. Erythrocyte folate (ng./ml. packed cells) was calculated from the whole blood folate using the formula of Mollin and Hoffbrand\textsuperscript{13}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Erythrocyte folate (ng./ml. packed cells) in control subjects, iron deficiency anemia and folate deficient anemia.}
\end{figure}
and the mean corpuscular folate (MCF) content was calculated from the following formula:

\[ MCF = \frac{\text{erythrocyte folate (ng./ml. of packed cells)}}{\text{Number of erythrocytes per ml. of packed cells}}. \]

**RESULTS**

**Erythrocyte Folate**

Figure 1 compares the erythrocyte folate values and shows the mean value and 95 per cent confidence limits for each of the three groups studied. The mean value for the control group was 183 ng./ml. packed cells. The folate-deficient patients had a mean value of 51 ng./ml. packed cells. For the iron-deficient group the mean was 336 ng./ml. packed cells. The differences between these groups are highly significant.

Figure 2 shows the MCF values for the control subject and the iron-deficient patients, as well as the mean value and 95 per cent confidence limits for each. The mean values in the two groups were, respectively, 1.71 ng. \( \times 10^{−8} \) and 2.62

![Fig. 2.—Mean corpuscular folate (ng. \( \times 10^{−8} \)) in control subjects and iron deficiency anemia.](image-url)
After treatment with oral iron.

Figures 3 and 4 show the erythrocyte folate and MCF values before and after treatment. The differences were significant at the five per cent level for erythrocyte folate, but not for the MCF values. In some cases, the erythrocyte folate fell to levels below the normal range.

Plasma Folate

There was no significant difference between the plasma folate values in the control subjects and the iron-deficient patients. The mean values and 95 per cent confidence limits were 4.34 (± 0.42) ng./ml. and 5.73 (± 1.57) ng./ml., respectively.
Figure 5 shows that in all but two cases there was a rise in plasma folate after treatment in the iron-deficient group. All values but one were, however, in the normal range.

DISCUSSION

The results presented confirm the findings of other investigators, that the red cell folate in megaloblastic anemia due to folate deficiency is very low.

There is also an abnormality in the distribution of blood folate between the red cells and the plasma in patients with iron deficiency anemia. The red cell folate (ng./ml packed cells) is significantly higher in these patients than in controls, while the plasma folate content is normal. Furthermore, if these results are expressed as the folate content of individual red cells the results are essen-
tially the same. In view of the fact that treatment with oral iron alone causes a reversion of the erythrocyte folate to normal, and as folate and iron are not metabolized by the mature red cell, it can be postulated that there is a failure of folate utilization in early red cells in iron deficiency. An analogous situation occurs in uncomplicated megaloblastic erythropoiesis due to either folate or vitamin B₁₂ deficiency, where iron accumulates in the developing red cells, giving rise to sideroblasts in the bone marrow, and, at times, the appearance of siderocytes in the peripheral blood. The interdependence of the metabolic functions of iron and folate is further shown in combined deficiency of these factors where treatment with either hemopoietic factor alone tends to precipitate the full picture of deficiency of the other, possibly due to increased utilization.
After treatment with oral iron, there was a rise in the plasma folate which was statistically significant at the five per cent level. However, as most levels were in the normal range both before and after treatment, it was difficult to interpret this, especially as the folate levels in the control subjects were not remeasured after a similar interval. However, it is interesting to note that three of the six iron-deficient patients reported by Velez et al.14 attained, on treatment with oral iron alone, a significant rise in the serum folate content. All patients had, in addition, associated megaloblastic changes which reverted to normal with iron therapy and the authors argued that iron deficiency may lead to secondary folate deficiency. This concept was suggested previously by Chanarin et al.15 who found a significant improvement in the folate status of iron-deficient females taking prophylactic iron therapy during pregnancy as judged by rises in their serum levels. However, Davidson16 has referred to a neutrophil shift to the right and the development of giant metamyelocytes in the marrow as a characteristic feature of iron depletion, and Beard and Weintraub17 have found that this was not due to folate depletion. They suggested that iron deficiency may impair the activity of formiminotransferase.

Summary

Erythrocyte and plasma folate levels were studied before treatment in 20 patients with iron deficiency anemia and in 23 patients with megaloblastic anemia due to folate deficiency. Fourteen of the cases of iron deficiency anemia were also studied after treatment with oral iron alone. Fifty-seven normal persons were used as controls.

The mean erythrocyte folate (ng./ml. packed cells) was significantly increased in iron deficiency anemia and significantly depressed in folate deficiency anemia. After treatment with oral iron alone, the mean erythrocyte folate level fell to normal in the iron deficiency anemia group. The mean corpuscular folate (ng. × 10^-8) was also significantly raised in iron deficiency: in eight of 10 cases this fell after treatment, but the overall fall was not significant. The plasma folate rose in iron deficiency anemia after oral iron treatment.

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