Effect of Long-Term Supplementation of Folate on Folate Status in Plasma and Erythrocytes

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Summary Folate nutritional status was estimated by radioassay of folate levels in plasma and erythrocytes during and after a long-term supplementation of folic acid. A 1-mg dose of folic acid per day was administered orally to 6 healthy subjects for 17 weeks. After 4 weeks of supplementation the mean folate concentration in plasma reached 11 ng/ml and remained constant thereafter, but decreased exponentially after stopping the supplementation. However, the folate concentrations in reticulocytes and erythrocytes increased linearly in all subjects during the supplementation. These results suggest that folate-rich, young erythrocytes are mixed at a constant rate with circulating ripe ones, which have a lower folate content, during folate supplementation.

Key Words folate, supplementation, plasma, erythrocyte, human, physiological doses, megadoses

Folate levels in plasma and erythrocytes are used in the assessment of folate status in various stages of folate deficiency in man, and cut-off points of the deficiency are well defined (1). Little information is available about the folate levels in plasma and erythrocytes during and after a long-term supplementation of folate. The folate concentration in plasma changes rapidly after folate intake (2). The folate concentration in erythrocytes reflects the folate status during erythropoiesis, because folate is only incorporated into the erythrocytes in this stage (3). Therefore, a single measurement of the folate concentration in erythrocytes may reflect the mean folate status during the various growing stages of the erythrocytes. The length of time required for erythrocytes to incorporate folate by adapting to various doses of folate has not been documented yet. In this paper a long-term study on folate supplementation was conducted. Folate concentrations in plasma and erythrocytes were measured during 17 weeks of supplementation, and for 7 weeks after the supplementation stopped.
Six healthy subjects (4 men and 2 women, aged 26 to 43 years) participated in this study. Folic acid (0.5 mg) was given two times daily in the morning and the evening for 17 weeks in addition to the normal German diet. In order to avoid any influence of recent folate absorption, a blood sample was taken in the morning from each subject after 12-h fasting and the sample was used to measure folate in plasma and erythrocytes according to the method of Mincey et al. (4) using a radioassay kit (Becton Dickinson folate radioassay kit).

RESULTS

All subjects showed a sufficient folate status above the cut-off levels of 3.0 ng folate/ml plasma and 160 ng/ml erythrocytes at the beginning of the study (Figs. 1 and 2). The results of plasma folate status are shown in Fig. 1. During the first 4 weeks of supplementation the mean level in plasma rose from a basal level of 5.5 ng/ml to 10.8 ng/ml. During the following 7 weeks the plasma level remained constant. Between the 11th and 15th weeks of supplementation an increase of 2 ng/ml was observed, whereas between the 15th and 17th weeks the level was 13 ng/ml. After supplementation stopped the concentration of folate in plasma fell rapidly in the 1st week and decreased slowly thereafter, but 7 weeks after cessation the level was down to 9.0 ng/ml, which was still higher than the basal level.

![Fig. 1. Effect of long-term folate supplementation (1 mg/day) on plasma folate concentration. Folate was supplemented for 17 weeks.](image)
Fig. 2. Effect of long-term folate supplementation (1 mg/day) on the erythrocyte folate concentration. Folate was supplemented for 17 weeks.

Fig. 3. Influence of folate supplementation on plasma folate concentration. Shaded area shows normal range of folate concentration.

Fig. 4. Influence of folate supplementation on erythrocyte folate concentration. Shaded area shows normal range of folate concentration.
The folate status of the erythrocytes of the 6 subjects are shown in Fig. 2. The levels increased linearly in all subjects during folate supplementation. The mean folate concentration rose from 252 ng/ml erythrocytes to 596 ng/ml, with an average rate of increase of 2.9 ng/ml per day. After supplementation stopped the level decreased linearly with a nearly constant rate of 3.2 ng/ml per day.

The influence of various folate doses on folate concentrations in plasma and erythrocytes, which was previously obtained in our studies (5), and data on very high doses of folate (25 to 1,000 mg) reported by Zettner et al. (6) are summarized in Figs. 3 and 4, where the normal physiological range of folate is indicated by shaded areas. The supplemented doses of folate per day are written near the curves.

Blood for all studies presented in this paper was taken in the morning in the fasting state 1 h before folate was given. One mg of folate was given in two doses per day: 0.5 mg in the morning and 0.5 mg in the evening. 0.43 mg and 25 mg up to 1,000 mg of folate were given once a day just before breakfast. The increase of folate levels in erythrocytes after various folate intakes are shown in Fig. 5.

DISCUSSION

Human intake of high doses of folic acid increases folate absorption from the intestine (7, 8). The daily addition of 1 mg of folate to the diet is generally referred to as a physiological dose (3). In this study the mean folate concentration in plasma reached a steady-state level during a 4-week period of folate supplementation. Folate binds to various proteins in the body fluids and tissues. The binding proteins are not completely saturated with folate under normal conditions (9). This may be
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the reason why 4 weeks were required in this study for a steady-state level of folate to be obtained.

The high level in plasma was maintained by continuous doses of folic acid. After folate supplementation stopped this high level could no longer be maintained and the plasma folate concentration decreased. As shown in Fig. 1 the folate concentration in plasma tends to decrease exponentially. Based on this decrease the half-time of folate elimination from plasma was calculated to be about 40 days. The folate levels continued to be above the basal levels in all subjects for 7 weeks after folate supplementation stopped. A lower dose of folate (0.43 mg/day) resulted in lower levels of plasma folate. Compared to the low-dose experiment, high doses of folate (>25 mg) resulted in an immediate and sharp increase in folate concentrations in plasma (Fig. 3). Since high doses of folate cannot be absorbed by body tissues, more than 50% of the supplemented folate were rapidly excreted in the urine (7).

The gradual increase in the folate concentration in erythrocytes due to long-term supplementation of folate can be explained by an increase in the ratio of young, folate-rich erythrocytes to circulating ripe ones that are less folate rich. This supports the concept that the incorporation of folate into precursor cells takes place only during erythropoiesis, as reported by Zettner et al. (6) Because of the continuous and constant exchange of ripe for young erythrocytes the folate level in erythrocytes increased weekly of about 20 ng/ml from 252 ng/ml to 596 ng/ml. After the supplementation of folate stopped its levels in erythrocytes decreased linearly since the supply of folate to the erythrocyte-producing tissues had been discontinued and young, less folate-rich erythrocytes were subsequently released into the circulation. No correlation exists between folate levels in plasma and erythrocytes, because on one hand the folate level in erythrocytes reflects long-term folate supply due to the incorporation of folate into reticulocytes. The plasma level, on the other hand, reflects the actual short-term supply of folate. Additionally, the exchange of incorporated folate is limited.

A similar increase in the folate concentration in erythrocytes was observed at all of the low-folate doses (0.43 mg, 0.90 mg, and 1.00 mg) as shown in Fig. 4. High doses up to 1,000 mg caused very high levels of folate in erythrocytes. The rapid increase in the folate concentration in erythrocytes due to long-term folate supplementation suggests that its incorporation into erythrocytes might be possible under these conditions. The differences between the increase in folate concentration after low and high doses of folate are shown in Fig. 5. This difference might be explained by penetration of folate into ripe erythrocytes after high-dose folate supplementation because of very high plasma levels.

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