STUDIES ON THE FOLIC ACID VITAMINS

VI. The Effect of Amethopterin on Erythropoiesis in Man

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DURING the course of an investigation of the effects of large doses of amethopterin in man,[3] it became apparent that the drug temporarily arrested production of the formed elements of the peripheral blood. The times at which the peripheral blood counts reached minimum values (reticulocytes 4.6 days, leukocytes 6.2 days, and platelets 9.3 days) bore a close relationship to the life spans of these elements as determined by various methods.[4, 7, 10] In order to gain more information about the effects of amethopterin on erythropoiesis, the present study was undertaken.

METHODS

Patients. Included were 1 woman with epidermoid carcinoma (primary unknown) metastatic to cervical lymph nodes, 1 woman with multiple basal cell carcinoma, 1 man with pseudomyxoma peritonei of appendiceal origin, and 1 man with hepatic metastases from recurrent adenocarcinoma of the kidney. All had normal peripheral hematological findings at the beginning of the studies, were ambulatory, and in good nutritional status.

Hematology. Microhematocrits of venous blood were done by the method of Strumia et al.[8] Reticulocyte and platelet counts were done by Brecher and co-workers' methods.[1, 2]

Isotope Techniques. Twenty milliliters of venous blood were obtained from the patient, mixed with 10 μc. of radioactive iron (Fe^{59}) citrate, and incubated for 30 minutes. The amount of iron added to the plasma was within the iron-binding capacity. A volume of blood containing approximately 8 μc. was reinjected. Venous blood samples were obtained frequently during the first few hours after injection to establish the rate of disappearance of the isotope from the plasma. Subsequent samples were collected at the times that the organs were scanned. The count rates over the liver, spleen, and sacrum (representing the bone marrow) due to Fe^{59} were measured by the method of Elmlinger et al.[5] Blood samples were measured in a well type scintillation counter with a single channel spectrometer to a maximum counting error of 3.5%. Correction was made for physical decay.

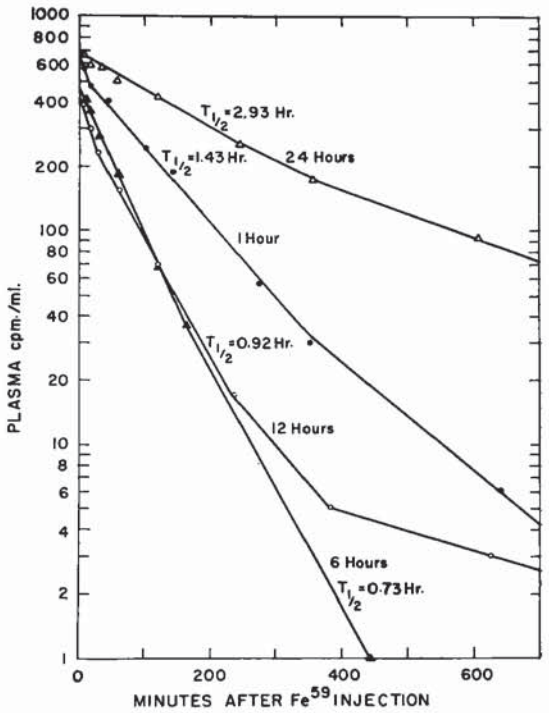

FIG. 1. Plasma Fe^{59} disappearance curves. The radioactivity in plasma, expressed as counts per minute per milliliter is plotted at different times after the injection of Fe^{59}. $T_{1/2}$ represents the half time for disappearance of Fe^{59} from the plasma and was determined graphically. The intervals between the administration of amethopterin and Fe^{59} are designated by 1, 6, 12, and 24 hours.

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RESULTS

Plasma Iron Disappearance. The plasma iron disappearance curves for 4 patients are presented in Fig. 1. When amethopterin was given 1 or 6 hours before the administration of Fe^{59}, the rate of disappearance of iron from the plasma was either within the range of normal or slightly greater than normal. With an interval of 12 hours between the administration of Fe^{59} and amethopterin, the initial disappearance curve had a half time of 0.92 hours. However, a marked change in the slope of the curve, greater than that usually observed in normal individuals, took place between about 7 and 10 hours after Fe^{59} administration or 19 and 22 hours after the administration of amethopterin. The fourth patient received Fe^{59} 24 hours after the administration of amethopterin and had an abnormally slow plasma iron disappearance curve. The half time of 2.93 hours, 2 to 3 times longer than normal, was nearly identical with the late portion of the plasma iron disappearance curve obtained when the interval between the administration of the drug and isotope was 12 hours.

One-Hour Interval. The results of giving Fe^{59} one hour after amethopterin are depicted in Fig. 2. The lower part of the figure shows the changes in the Fe^{59} content of the liver, spleen, and sacrum corrected for the isotope contained in the vascular system. The counts over the sacrum increased rapidly to a maximum value the first day and thereafter decreased rapidly to nearly 0 by day 18. The liver and spleen both showed an initial increase in activity, in each case considerably less than the sacrum, followed by a slow disappearance with time. The appearance of radioactivity in erythrocytes is shown in the upper part of the figure. The incorporation proceeded rapidly, reaching 50% on day 5 and 80% on day 12 after Fe^{59} administration. The reticulocytes decreased, reaching a minimum value of 0.1% on day 3, while recovery became apparent on day 8. Changes in hematocrit were erratic but represented some decrease in the initial value of 39.5%. A leukocyte count of 2,600 cells per cu. mm. on day 8, and a platelet count of 168,000 per cu. mm. on day 12 suggest a moderate drug effect. Oral ulcerations were not seen in this patient.

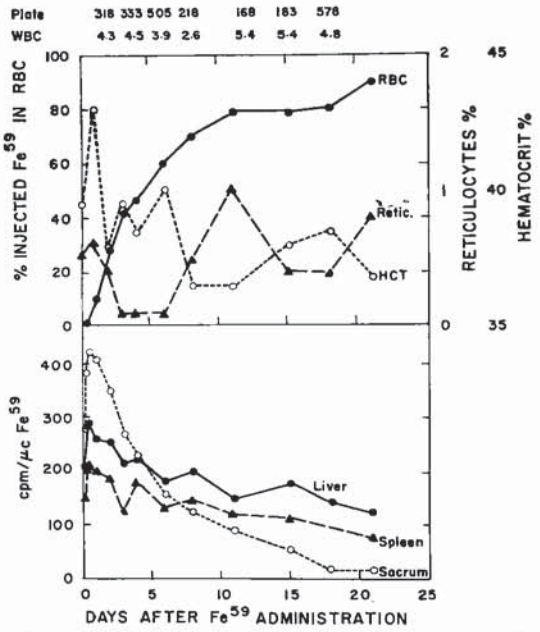

FIG. 2. Patient S.K., a 55-year-old white woman with multiple basal cell carcinomas, who had received 8 μc. of Fe^{59} 1 hour after 10 mg. per kg. (800 mg.) of amethopterin. The following abbreviations are used: plate, platelets per cubic millimeter times 10^{-3}; WBC, leukocytes per cubic millimeter times 10^{-3}.

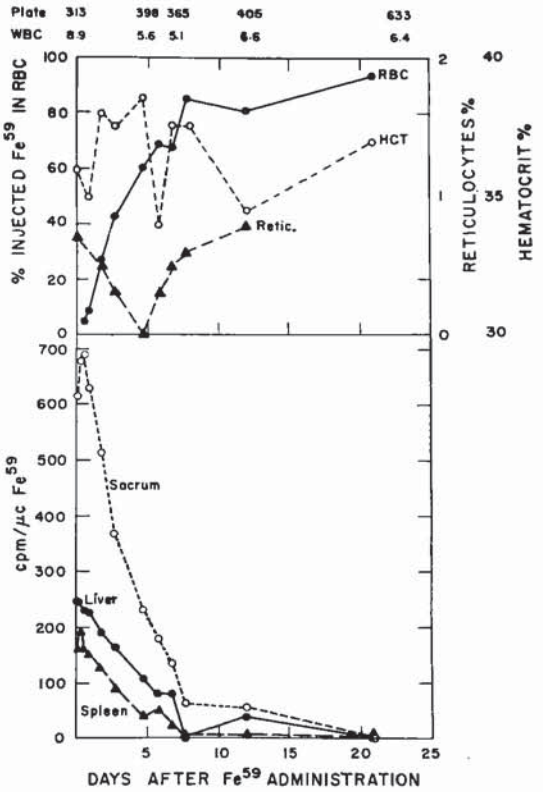

FIG. 3. Patient H.H., a 49-year-old white man with pseudomyxoma peritonei, who had received 8 μc. of Fe^{59} 6 hours after 14 mg. per kg. (925 mg.) of amethopterin. For explanation of the abbreviations, see Fig. 2.

Six-Hour Interval. The results of giving Fe^{59} 6 hours after amethopterin are recorded in Fig. 3. The changes in isotope content of the viscera and its incorporation into peripheral erythrocytes were similar to those observed after the 1-hour interval. Reticulocytes decreased to 0 on day 4 and recovered promptly. Changes in hematocrit were not consistent. A leukocyte count of 5,100 cells per cu. mm. recorded on day 7 was somewhat below the initial count of 8,900 cells per cu. mm. but does not indicate serious drug effect. No reduction in platelet count was observed, but the value of 633,000 per cu. mm. recorded on day 23 may represent a rebound from the effect of amethopterin.

Twelve-Hour Interval. The results of waiting 12 hours after amethopterin before giving Fe^{59} are presented in Fig. 4. Again the changes in isotope distribution are similar to those observed after the 1-hour interval. Reticulocytes decreased to 0 on day 4, after which they slowly recovered. The hematocrit once again showed considerable variation from day to day and in general showed some decrease.

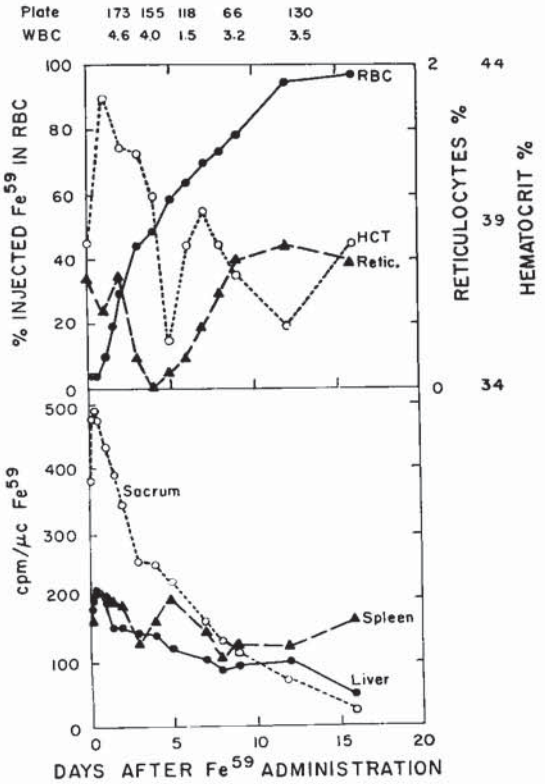

FIG. 4. Patient J.H., a 53-year-old white man with renal carcinoma, who had received 8 μc. of Fe^{59} 12 hours after 10 mg. per kg. (630 mg.) of amethopterin. For explanation of the abbreviations, see Fig. 2.

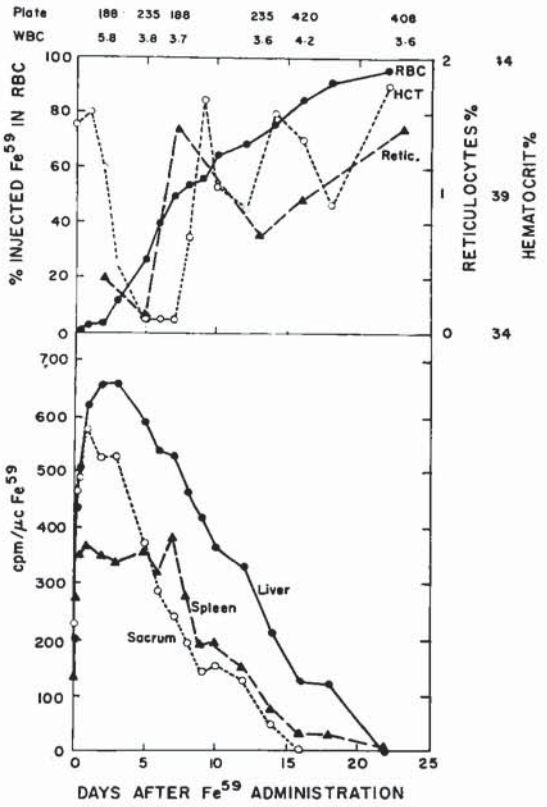

FIG. 5. Patient E.R., a 39-year-old white woman with metastatic carcinoma, who had received 8 μc. of Fe^{59} 24 hours after 12 mg. per kg. (750 mg.) of amethopterin. For explanation of the abbreviations see Fig. 2.

The minimum value of 1,500 cells per cu. mm. for the leukocytes obtained on day 6 and of 66,000 per cu. mm. for the platelet count on day 9 indicated a marked drug effect in this patient, but no oral ulcerations were present.

Twenty-four-Hour Interval. The results of waiting 24 hours before giving Fe^{59} after amethopterin are shown in Fig. 5. As shown in the lower part of the figure, the counts over the sacrum reached a maximum value on the first day but were sustained for 2 additional days before a decrease occurred. The activity over the liver rose rapidly to values greater than those obtained over the sacrum, were maintained for about 3 days, and then declined. Counts over the spleen were less than those over the sacrum or liver and remained essentially constant for 8 days, after which they decreased steadily to 0 by day 22. As shown in the upper part of the figure, incorporation of Fe^{59} into erythrocytes did not begin until the third day after Fe^{59} administration. Fifty per cent of the incorporation

occurred on day 7, and maximum values of about 90% incorporation occurred on day 18. Reticulocytes decreased to 0.2% on day 5 and then promptly recovered. Hematocrit decreased from 42% to 35% by day 5 and had recovered to 42% by day 9. Leukocytes reached a minimum value of 3,700 cells per cu. mm. on day 7 and were still depressed at the end of the period of observation, 23 days after Fe^{59} administration. Platelet counts showed little change during the first 13 days, but a moderate rise occurred subsequently.

DISCUSSION

The previous study of the effects of large doses of amethopterin indicated that this drug caused a temporary decrease in the number of cells in the peripheral blood.[3] The time that elapsed after the administration of amethopterin before the reticulocyte counts reached minimum values (4.6 days) corresponded closely to the maturation time of transfused reticulocytes (5 to 6 days)[10] and suggested that the decrease was due to the normal maturation of reticulocytes at a time when no new cells were being liberated from the marrow. Such an arrest in the production of erythrocytes should be reflected by alterations in the distribution into liver, spleen, and bone marrow of injected Fe^{59} and its incorporation into red cells. When Fe^{59} was given 24 hours after the administration of a single dose of amethopterin, abnormal patterns of isotope distribution were obtained. The rate of disappearance of Fe^{59} from the plasma was slower than normal by a factor of 2 or 3; the uptake of Fe^{59} by the liver was greater than that of the bone marrow; the activity in the liver and sacrum remained approximately constant for 3 days and in the spleen for 8 days; at day 3 for the liver and sacrum and day 8 for the spleen, the activity of all 3 organs decreased at nearly the same rate. Little or no incorporation of isotope into peripheral erythrocytes occurred until the third day after Fe^{59} administration, which was the fourth day after the administration of amethopterin. Once begun, however, the appearance of labeled erythrocytes proceeded in a normal manner, 50% incorporation being achieved at about 4 days after production began or 7 days after Fe^{59} administration. Thus in patient E.R., Fe^{59} was stored in the marrow, represented by the sacrum, and liver for about 3 days and the spleen for 8 days, and then utilized for erythropoiesis. Fe^{59} was retained in the liver and spleen until the bone marrow had begun the production of erythrocytes. Even though the Fe^{59} was presumably carried in the plasma between the liver and sacrum after erythropoiesis had resumed, it was not possible to demonstrate any increase in the concentration of Fe^{59} in the plasma during this period of time because of rapid clearance by the marrow and inability to measure extremely low concentrations of isotope.

When shorter intervals of 1 or 6 hours intervened between the administration of amethopterin and Fe^{59}, no alteration in the disappearance of Fe^{59} from the plasma or in the appearance of labeled erythrocytes could be detected. The accumulation in the various organs immediately after the injection of Fe^{59} and the subsequent release took place in a normal manner.

An interval of 12 hours between amethopterin and Fe^{59} administration also produced a normal pattern of storage and release of Fe^{59} by the various organs, as well as the appearance of labeled erythrocytes in the peripheral blood. However, the disappearance of Fe^{59} from the plasma suggested that a change took place 7 to 10 hours after the administration of Fe^{59}. The half time of Fe^{59} disappearance during the first 6 hours after injection was slightly less than 1 hour, or within the range of normal. An abrupt change in slope occurred after this time, representing a much slower rate of elimination of Fe^{59} from the plasma. This change in the rate of disappearance occurred 19 to 22 hours after amethopterin administration, which is consistent with the data obtained for patient E.R.—that 24 hours after amethopterin there was diminished uptake of Fe^{59} by the marrow.

If it is assumed that amethopterin reaches the bone marrow in physiologically active concentration rapidly (i.e., minutes) after administration, then the results suggest that the inhibition of erythrocyte production by amethopterin takes place before iron is incorporated into the developing cell. Since Fe^{59} has been shown to enter pronormoblasts[9] and to be utilized for hemoglobin synthesis in these young cells,[6] amethopterin may act at the earliest stage in erythrocyte production. It is also apparent that an interval of 20 to 24 hours is required for the cell to mature from the site of action of amethopterin to the stage at which Fe^{59} is incorporated. At the doses employed in this study, this inhibition appears to be reversible.

CONCLUSIONS

1. Amethopterin temporarily inhibits incorporation of radioactive iron in erythrocytes.
2. In order to achieve this effect, amethopterin must be given at least 20 hours before the administration of Fe^{59}.
3. The method employed in this study can be utilized to determine the effect of drugs upon erythropoiesis.

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