Megaloblastic Anemia of Pregnancy in Two Sisters
Case Reports and an Investigation of the Hemolytic Mechanism by Means of Erythrocyte Survival Studies

By James W. Hollingsworth and Fae M. Adams

Megaloblastic Anemia is a rare complication of pregnancy, and the occurrence of the disease in members of the same family has not been noted. We recently had the opportunity to observe two sisters with this disease at Walter Reed Army Medical Center, and the studies performed on these patients are the basis of the present report.

Materials and Methods
Blood studies were done by the standard methods employed in our laboratory. In the determinations of erythrocyte lifespan with radioactive chromium, 30 ml. of blood in acid-citrate-dextrose solution (ACD) were incubated for one hour with 100 microcuries of Na_2CrO_4 (Abbott). The cells were then washed once in isotonic saline and injected into normal recipients of compatible blood group. Five ml. blood samples from the recipients were taken at intervals and the radioactivity measured in a well-type scintillation counter. Counting times were selected to insure a random sampling error of less than 5 per cent.

Report of Cases

Case 1, a 20 year old white woman, was seen in the Prenatal Clinic at Walter Reed Army Hospital on September 23, 1953, in the fourth month of her first pregnancy. She was in good health and routine blood examination ten days later revealed hgb. 10.1 Gm. per cent, hematocrit 35 ml. per 100 ml., red blood cells 3,000,000 per cu. mm. and white blood cells 6,500 per cu. mm. The morphology of the leukocytes was normal. The patient was not seen again until she was admitted to the hospital on January 29, 1954, complaining of crampy abdominal pain and vomiting for a week, and slight vaginal bleeding for two weeks. Physical examination revealed pallor but no jaundice. The tongue showed no atrophy of the papillae. A grade 1 systolic murmur was heard over the pulmonary area. The uterus was enlarged almost to the xyphoid process; liver and spleen were not palpable. On pelvic examination a chronic cervicitis was found to be the cause of the vaginal bleeding. Blood examination revealed hgb. 6.9 Gm. per cent, red blood cells 2,390,000 per cu. mm. and hematocrit 21 ml. per 100 ml. Serum bilirubin, on February 8, was 1.2 mg. per cent with 0.8 mg. direct reacting. Because of the anemia blood transfusion of 1000 ml. was administered on the second hospital day. Bone marrow aspiration, performed on the ninth hospital day, revealed very cellular marrow with typical megaloblasts, giant metamyelocytes and inadequate platelet production by the megakaryocytes. Examination of the blood smear revealed moderate anisocytosis with some macrocytes, decreased platelets and leukocytes, an occasional orthochromatic megaloblast and rare multilobed polymorphonuclear leukocytes. Therapy with folic acid, 20 mg. daily by mouth, was begun and continued in the hospital for 10 days. The gastro-intestinal symptoms subsided and the patient felt well. Changes in the findings of laboratory studies after treatment are recorded in table 1. She was discharged from the hospital with instructions to take 10 mg. of folic acid orally each day, and to be seen periodically as an out-patient. On March 28, she delivered a normal male infant and left the hospital two days later. At the time of delivery the blood smear had reverted to normal. A gastric analysis revealed copious quantities of gastric juice con-
MEGALOBLASTIC ANEMIA OF PREGNANCY IN TWO SISTERS

Table 1.—Case 1. Response to Therapy with Folic Acid

<table>
<thead>
<tr>
<th>Date</th>
<th>Days of Folic Acid Therapy</th>
<th>Hgb. (Gm.)</th>
<th>RBC (X10⁶)</th>
<th>Hema-</th>
<th>M.C.V. (cu. microns)</th>
<th>Retic. (%)</th>
<th>Platelets</th>
<th>Leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 Jan.</td>
<td>-12</td>
<td>6.9</td>
<td></td>
<td>21</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>31 Jan.</td>
<td>-10</td>
<td>7.1</td>
<td>2.5</td>
<td>23</td>
<td>92</td>
<td>1.7</td>
<td>44,000</td>
<td>3,100</td>
</tr>
<tr>
<td>9 Feb.</td>
<td>0</td>
<td>7.1</td>
<td>2.4</td>
<td>22</td>
<td>92</td>
<td>1.7</td>
<td>49,000</td>
<td>6,200</td>
</tr>
<tr>
<td>12 Feb.</td>
<td>4</td>
<td>8.9</td>
<td>3.3</td>
<td>28</td>
<td>85</td>
<td>8.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15 Feb.</td>
<td>7</td>
<td>9.3</td>
<td>3.3</td>
<td>27</td>
<td>82</td>
<td>11.6</td>
<td>213,000</td>
<td>7,700</td>
</tr>
<tr>
<td>17 Feb.</td>
<td>9</td>
<td>9.7</td>
<td>3.6</td>
<td>33</td>
<td>92</td>
<td>6.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>19 Feb.</td>
<td>11</td>
<td>9.7</td>
<td>3.6</td>
<td>33.5</td>
<td>93</td>
<td>6.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>25 Feb.</td>
<td>17</td>
<td>10.1</td>
<td>3.9</td>
<td>34.5</td>
<td>89</td>
<td>4.4</td>
<td>261,000</td>
<td>11,600</td>
</tr>
<tr>
<td>5 Mar</td>
<td>22</td>
<td>10.6</td>
<td>3.8</td>
<td>35.0</td>
<td>92</td>
<td>3.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10 Mar</td>
<td>30</td>
<td>10.0</td>
<td>4.0</td>
<td>33.5</td>
<td>84</td>
<td>4.8</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

... taining free hydrochloric acid. She did not return for follow-up examination, but a letter received four months later reported that she was working and had no symptoms.

Case 2, the 22-year-old sister of the first patient, was admitted to the hospital for delivery of her first child on February 23, 1954. She had been seen before delivery in the Prenatal Clinic on several occasions. On her last visit on January 6, a routine blood examination revealed hgb. 10.8 Gm. per cent, red blood cells 3,700,000 per cu. mm. and white blood cells 6,750 per cu. mm. She had noted no symptoms before admission to the hospital and labor and delivery were normal. Three days later, as she was awaiting discharge from the hospital, blood examination showed hgb. 5.8 Gm. per cent and hematocrit of 18 ml. per 100 ml. She was given 1000 ml. of blood by transfusion at that time. Abnormal physical findings on March 1, were limited to moderate pallor and fever of 100 F. which had been present since delivery. No abnormalities of the tongue or splenomegaly were noted. Blood examination in our laboratory demonstrated the anemia, moderate thrombocytopenia and leukopenia. Bone marrow aspiration revealed typical megaloblastic changes. Treatment with folic acid, 20 mg. daily by mouth, was started on March 2. The patient left the hospital the next day, to be followed as an out-patient. Examinations of the blood before and after therapy are summarized in Table 2. Two weeks after treatment was started, she moved to Florida and a letter received six weeks later stated that she felt well and that blood examinations by her physician were normal. She had stopped folic acid therapy approximately six weeks after delivery.

Family History: The patients were of mixed Scotch-Irish and French ancestry. There was no family history of anemia, jaundice or gallstones, and three grandparents had lived

Table 2.—Case 2. Response to Therapy with Folic Acid

<table>
<thead>
<tr>
<th>Date</th>
<th>Days of Folic Acid Therapy</th>
<th>Hgb. (Gm.)</th>
<th>RBC (X10⁶)</th>
<th>Hema-</th>
<th>M.C.V. (cu. microns)</th>
<th>Retic. (%)</th>
<th>Platelets</th>
<th>Leukocytes</th>
</tr>
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<td>-3</td>
<td>5.8</td>
<td></td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 Feb.</td>
<td>-2</td>
<td>given 1000 ml. of normal whole blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Mar.</td>
<td>0</td>
<td>9.3</td>
<td>3.0</td>
<td>29</td>
<td>97</td>
<td>0.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2 Mar.</td>
<td>1</td>
<td>9.6</td>
<td>3.1</td>
<td>30</td>
<td>97</td>
<td>4.2</td>
<td>174,000</td>
<td>7,900</td>
</tr>
<tr>
<td>5 Mar.</td>
<td>4</td>
<td>11.0</td>
<td>3.4</td>
<td>35</td>
<td>103</td>
<td>6.1</td>
<td>197,000</td>
<td>7,900</td>
</tr>
<tr>
<td>8 Mar.</td>
<td>7</td>
<td>11.3</td>
<td>3.8</td>
<td>37</td>
<td>97</td>
<td>7.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10 Mar.</td>
<td>9</td>
<td>11.2</td>
<td>3.8</td>
<td>36</td>
<td>95</td>
<td>4.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>12 Mar.</td>
<td>11</td>
<td>12.1</td>
<td>3.95</td>
<td>38</td>
<td>96</td>
<td>3.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>17 Mar.</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
to old ages; the fourth had died suddenly of unknown cause. The patient's mother had delivered five children without complications, and all members of the family were in good health.

**Dietary History:** Both sisters ate meat at least once daily during the entire pregnancy, and had ample quantities of fruits and vegetables. Although both were living in Washington and visited together almost daily, they rarely ate together.

**Special Studies**

**A. Erythrocyte Lifespan:** Erythrocytes from case 1 were tagged with radioactive chromium and given to normal recipients before treatment and at 10 and 48 days after administration of folic acid was started. Similarly cells from patient 2 were given to normal recipients before treatment and again ten days later. The results, plotted in figures 1 and 2, were similar in both cases. Radioactivity of the cells obtained before treatment declined at a normal rate for 20-25 days, and then rapidly and totally disappeared from the circulation of the recipients. Cells from the patients 10 days after treatment exhibited a similar disappearance, except that a small percentage remained after the sharp drop, and decreased at a slow rate. Cells from patient 1, obtained and transfused 48 days after therapy, showed a rapid drop in radioactivity during the first week, and a normal rate of disappearance thereafter.

Both patients had been transfused with 1000 ml. of normal blood before the initial sample was taken. Patient 1 had received blood 10 days before the sample was taken, and patient 2 had been transfused only two days before. Since the patients were very anemic at the time of transfusion, the normal cells should have constituted at least one-fourth of their circulating red cells. Note that in the survival curves of the first trial in each case there was no evidence of any cells, normal or other, surviving in the recipients after 20 to 40 days.

**B. Search for antibodies causing the unusual erythrocytes survival curves:** One explanation for the sudden disappearance of the patients' red cells from the blood of the normal recipients is the development of an antibody to the donor cells in the recipient. This type of delayed lysis of incompatible cells has been described in Rh incompatibility. Search for antibodies in the serum of recipients 1 and 2 of cells from patient 1 were undertaken. In our laboratory serum from recipient 1 obtained 30 days after injection caused a weak agglutination of fresh albumin-suspended cells from the donor (case 1). A further attempt at detection and identification of antibodies was undertaken by Dr. Hugh Chaplin at the National Institutes of Health. The 30-day serum from recipient 1 caused a 2-plus conglutination reaction at 37 C. with the donor's fresh cells suspended in their own serum. At room temperature the donor cells, both in saline and after sensitization with trypsin, were strongly agglutinated by the recipients' serum. At the same time, however, no agglutination occurred with recipient serum and donor cells in albumin suspension at 37 C., and the indirect Coombs reaction was negative.

Two weeks later serum from recipients 1 and 2, similarly tested against fresh cells from case 1, was entirely negative. Red cells from other cases of untreated megaloblastic anemias were not available for testing. Genotyping (C, D, E, c, e, M, N, P, Fy*, Kell) of the donor and the two recipients revealed no antigen present in the donor and lacking in both of the recipients. The serum from recipient 1, obtained 30 days after transfusion, was negative against Dr. Chaplin's...
cell panel (definitive for C, D, E, c, e, M, N, S, P, Le, Fy, and Lu). In summary, no demonstrable antibody to known blood group antigens present in the erythrocytes of case 1, developed in two recipients. The initial agglutination of the donor cells by serum from recipient 1 was not explained.

**Discussion**

Our two cases represent the first reported instance of familial occurrence of megaloblastic anemia of pregnancy. There seems no reasonable doubt of the diagnosis in these two sisters. The blood examinations revealed a pancytopenia with some macrocytic erythrocytes, although the mean corpuscular volume was normal in one case and only slightly increased in the other. Similar findings have been reported in other cases of megaloblastic anemia of pregnancy, and the variability of the mean corpuscular volume has been stressed. Examination of the bone marrow of both patients revealed the typical changes of a megaloblastic anemia. Addisonian pernicious anemia, which frequently exhibits familial occurrence, was excluded by the lack of achlorhydria in our first patient. Our two patients responded well to oral administration of folic acid, as have other patients with this disease.

The basic etiology of this megaloblastic anemia occurring during pregnancy, although presumably due to folic acid deficiency, has not been determined. In women with hereditary hemolytic diseases, the increased erythropoiesis accompanying the hemolytic syndrome plus the increased demands of pregnancy might lead to folic acid deficiency. Indeed, two instances of megaloblastic anemia of pregnancy accompanying hereditary hemolytic diseases have been described. The familial occurrence of megaloblastic anemia in our patients, then, might logically be explained if both patients had a familial hemolytic disorder, but there is no evidence of pre-existing disease in our patients and treatment with folic acid produced an apparent cure of the anemia.

No studies of the hemolytic aspects of megaloblastic anemia of pregnancy have been reported, although Thompson and Ungley mentioned rapid hemolysis of normal erythrocytes by three of their cases. In patients with pernicious anemia, however, a number of studies of red cell lifespan by the Ashby differential agglutination method have been performed. Singer, King and Robert and Louttit gave cells from patients with untreated pernicious anemia to normal subjects, and found a diminished lifespan. These observations led to the conclusion that the hemolytic aspect of pernicious anemia is due to intrinsic defect of the cells. Mollison's observations that normal erythrocytes were not excessively destroyed by patients with pernicious anemia tended to confirm the belief that the hemolytic process in patients with pernicious anemia is due to intracorpuscular abnormality.

Recently, however, Hamilton and his co-workers observed a distinct shortening of the lifespan of normal cells given to patients with pernicious anemia. Specific therapy with vitamin B did not effect the hemolysis of normal blood transfused shortly before institution of therapy, but the extrinsic hemolytic factor disappeared in one patient nine days after B therapy, as evidenced by normal lifespan of transfused erythrocytes.

The results of our studies of the disappearance in normal recipients of Cr.
labeled cells from our patients are difficult to interpret. The radiochromium method for estimation of erythrocyte lifespan is relatively new, and the binding of the isotope to the hemoglobin of the cells is not absolute. Some of the isotope disappears from normal circulating cells by elution.\textsuperscript{18} Allowing for the elution from the erythrocytes, however, the technic has proved reliable in the estimation of lifespan of normal cells,\textsuperscript{19} and in studies of a variety of hemolytic diseases.\textsuperscript{19} The reliability of this isotopic method in studies of megaloblastic anemias has not been determined by comparison with the Ashby technic.

However, we do feel that certain conclusions concerning the validity of our observations can be drawn from the studies, largely because both patients had received 1000 ml. of normal blood shortly before the experiments were started. The so-called “patients’” cells obtained before treatment with folic acid actually consisted of a mixture of autogenously produced cells and cells obtained by the transfusions. Estimating from the increase in hematocrit of the two patients following transfusion, at least 25 per cent of the cells in this first sample given to normal recipients were those obtained from the transfusions. When this heterogeneous blood was tagged with Na\textsubscript{2} Cr\textsuperscript{51} O\textsubscript{4} and injected into normal recipients, no circulating tagged cells were found in the recipients after 4-6 weeks (figs. 1

![Graph](image)

**Fig. 1.—Results of transfusion of Cr\textsuperscript{51} tagged erythrocytes form patient 1 into normal subjects.**
MEGALOBLASTIC ANEMIA OF PREGNANCY IN TWO SISTERS

Fig. 2.—Results of transfusion of Cr²⁺ tagged erythrocytes from patient 2 into normal subjects.

and 2), although by the counting times employed as little as 2 per cent of the initial radioactivity would have been detected.

The normal cells transfused into the patients before the studies were started, then, were not demonstrable in the recipients. Since the behavior of Cr²⁺-tagged normal cells in normal recipients is well established, it seems likely that the normal erythrocytes in our two patients were changed in such a way that their life-span became identical with that of the autogenously produced megaloblastic erythrocytes.

When blood was taken from the two patients 10 days after institution of therapy with folic acid, the survival curve in normal recipients was almost identical with that obtained before treatment, except for the appearance of a small percentage of radioactivity remaining after most of the activity had disappeared (figs. 1 and 2). In case 1, cells were transfused to a normal recipient 45 days after treatment had begun. The survival of these cells was distinctly different, with a rapidly decreasing component evident during the first ten days, followed by a slower component disappearing at an approximately normal rate (fig. 1).

If the results obtained do represent true disappearance of cells, rather than some alteration in the rate of Cr²⁺ elution, the peculiar shape of the initial sur-
vival curves requires explanation. These curves are similar in shape to those observed due to active antibody production in the recipient, but no antibody was demonstrated by careful study. Sudden lysis of the cells might occur due to a sudden breakdown of some essential metabolic system of the cell, but no such mechanism has been demonstrated. Another possible explanation for the shape of the curves might lie in unequal blood production during the period of change from normoblastic to megaloblastic erythropoiesis, with subsequent unequal curves of cell destruction.

Presuming that the Cr⁴¹ disappearance is representative of cell lysis, certain tentative conclusions can be drawn from our data. It is evident that normal erythrocytes when transfused to our patients became damaged, and the damage remained even after the transfused cells were reintroduced into a normal subject. The observations made after 10 days of folic acid therapy suggested that cells with normal lifespan had reappeared, but the majority of the cells retained their abnormal lifespan. The one observation after 45 days in case 1 demonstrates a larger component of cells with a normal lifespan, but the initial rapidly falling cell component is not readily explained. This patient did remain pregnant, and it is possible that her disease was not entirely corrected by the therapy she received.

In general, our data fit the hypothesis that the basic hemolytic defect in these two patients was caused by an extrinsic factor permanently damaging all cells, either those endogenously produced or those received by transfusion. After folic acid therapy, this extrinsic factor apparently disappeared. Such a hypothesis is compatible with the data obtained by Hamilton and his co-workers in patients with pernicious anemia. However, it should be stated that our results may represent only some unexplained inadequacy of the radio chromium method of studying erythrocyte lifespan in patients with megaloblastic anemia.

SUMMARY

1. Two sisters with megaloblastic anemia of pregnancy were reported.
2. Erythrocytes from the patients, both before and after therapy with folic acid, were tagged with radioactive sodium chromate and injected into normal subjects. The results of these studies were discussed.

SUMMARIO IN INTERLINGUA

1. Es reportate le casos de duo sorores con anemia megaloblastic de pregnantia.
2. Erythrocytos del patientes—tanto ante como etiam post therapia a acido folic—esseva etiquettate con chromato de nat.rium radioactive e postea injicie a in subjectos normal. Le resultatos de iste studios es discutite.

REFERENCES

MEGALOBLASTIC ANEMIA OF PREGNANCY IN TWO SISTERS

Megaloblastic Anemia of Pregnancy in Two Sisters: Case Reports and an Investigation of the Hemolytic Mechanism by Means of Erythrocyte Survival Studies

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