Refractory Megaloblastic Anemia Associated with Excretion of Orotic Acid

By Charles M. Huguley, Jr., James A. Bain, Shirley L. Rivers and Robert B. Scoggins

The majority of anemias associated with a megaloblastic bone marrow respond to vitamin B₁₂ parenterally. Those which do not respond usually fall into well defined categories of nutritional deficiency, intestinal malabsorption syndromes, pernicious anemia of pregnancy, megaloblastic anemia of infancy or reactions to certain drugs. When these can be excluded a few cases remain which have been described as "idiopathic megaloblastic anemia." This is probably identical with the "achrestic anemia" of Israels and Wilkinson. The rarity of such "idiopathic" cases is illustrated by the findings of only 14 instances in a total of 577 cases of megaloblastic anemia by Davidson. It is important to note that all the types of vitamin B₁₂ refractory megaloblastic anemia listed above, including the idiopathic type, respond to the administration of folic acid. Nevertheless, an occasional case is encountered which does not fall into any of these categories and which is also refractory to treatment with folic acid as well as with vitamin B₁₂.

We are here reporting such a case, an infant with a severe anemia characterized by hypochromic erythrocytes and a megaloblastic bone marrow. No response followed treatment with vitamin B₁₂ or folic acid by mouth or by injection. The anemia was likewise refractory to pyridoxine and to uracil. Nevertheless, there was a partial remission following adrenal steroid therapy, although megaloblasts persisted in the marrow. Finally there was an excellent response to a mixture of uridylic and cytidylic acids administered while steroids were continued. This remission persisted for a time after withdrawal of steroids.
A striking feature of the illness was the passage in the urine of large amounts of orotic acid, an important intermediary of pyrimidine metabolism.

**Case Report**

J.M.R., a white male infant, was born on June 19, 1954 at full term. Delivery was normal. Birth weight was 8 pounds, 9 ounces. He was placed on SMA formula. Health and development seemed quite normal, except that he seemed to his mother to be less active and more somnolent than his older siblings. At three months of age his mother noted pallor. At four months he developed coryza, and a cough which persisted. At five months he began to have a diarrhea with large, loose, pale, foul-smelling stools. He was hospitalized and found to have a severe anemia with a red blood count of 2.55 million per cu.mm. and a hemoglobin concentration of 6.0 Gm.%. He was given antibiotics and was transfused. Two weeks later he was readmitted because of persistence of diarrhea and cough. He was placed on a moderately low-fat milk preparation, Prosana. Subsequent stools seemed normal. He ate egg yolk, meats, fruits and vegetables. He was given iron and vitamin preparations (Polyvisol, 0.6 ml. and Zymatinic, 4 ml. daily), so that he received 250 mg. ferrous gluconate, 50 mg. ascorbic acid, 4 mg. folic acid, 4 µg. vitamin B₁₂, and 260 mg. liver concentrate daily. He took these preparations from that time until his final admission. For about two months he improved, but in mid-January, 1955, he again developed an upper respiratory infection with cough. His hemoglobin was found to be 9.0 Gm. per cent. Despite antibiotics his symptoms continued and the anemia worsened. On March 14, 1955, at age 9 months, he was admitted to Emory University Hospital for the first time. The infections had been treated with Gantrisin, penicillin and tetracycline. He had received no chloramphenicol nor anti-convulsant drugs.

The parents were living and well. There was no family history of anemia or jaundice. The parents had a common ancestor, believed to be French in origin, who was the grandfather of the father and the great-grandfather of the mother. All other ancestors were English or Scotch. Three siblings were well. Neither parent, nor any other sibling, had any anemia or abnormalities of the erythrocytes.

On physical examination the child appeared pale and weak, but otherwise well developed and well nourished. Activity seemed subnormal. The sclerae were definitely blue in color. There was no jaundice. The skin was clear except for a few petechiae about the neck. The tongue had a normal coat. There was no enlargement of the lymph nodes. A few rales and rhonchi were scattered through the lung fields. The heart appeared normal except for a soft apical systolic murmur. The edge of the liver was just palpable. The tip of the spleen descended 1 to 2 cm. on inspiration. No neurologic abnormalities were noted. There was torsion of the left tibia outward. There were no other skeletal abnormalities.

Laboratory findings included the following: erythrocytes, 2.80 million per cu.mm.; hemoglobin, 6.7 Gm. per cent; reticulocytes, 2.2 per cent; platelets, 280,400 per cu.mm.; WBC, 2,050 per cu.mm. Differential white blood cell count revealed 44 per cent segmented neutrophils, 29 per cent lymphocytes and 27 per cent monocytes. The appearance of the erythrocytes was very abnormal (fig. 1). There was pronounced anisocytosis with many macrocytes and many microcytes. The cells were quite hypochromic, and there was a considerable degree of poikilocytosis. There were two nucleated RBC per 100 WBC. No hematocrit determination was made, and the only red cell index calculated was the mean corpuscular hemoglobin, which averaged 21.3 µg.

The marrow was hypercellular with striking abnormalities of the pernicious anemia type in cells of both granulocytic and rubricytic series. All differential marrow counts are tabulated in table 1.

Serum iron was 200 µg. per cent. The serum direct bilirubin was 0.25 mg. per cent, and the total bilirubin was 1.3 mg. per cent. Coomb's test was negative.

A specimen of urine had a pH of 5.5 and specific gravity of 1.021; albumin and sugar...
MEGALOBLASTIC ANEMIA AND OROTIC ACID EXCRETION

Table 1.—Bone Marrow Differential Counts

<table>
<thead>
<tr>
<th></th>
<th>3-16-55</th>
<th>3-26-55</th>
<th>5-2-55</th>
<th>6-20-55</th>
<th>7-29-56</th>
<th>7-31-56</th>
<th>8-11-56</th>
<th>10-17-56</th>
<th>12-26-56</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYELOBLASTS</td>
<td>5.3</td>
<td>2.7</td>
<td>0.7</td>
<td>1.0</td>
<td>0.3</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>PROMYELOCTES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEUTROPHILS:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYELOCYTES</td>
<td>4.3</td>
<td>4.0</td>
<td>7.3</td>
<td>8.0</td>
<td>8.7</td>
<td>12.6</td>
<td>11.7</td>
<td>7.3</td>
<td>15.0</td>
</tr>
<tr>
<td>METAMYELOCYTES</td>
<td>10.0</td>
<td>6.0</td>
<td>12.7</td>
<td>4.0</td>
<td>4.0</td>
<td>8.0</td>
<td>2.7</td>
<td>5.0</td>
<td>4.0</td>
</tr>
<tr>
<td>BANDS</td>
<td>1.0</td>
<td>1.7</td>
<td>5.3</td>
<td>6.3</td>
<td>2.0</td>
<td>7.0</td>
<td>10.7</td>
<td>10.3</td>
<td>12.7</td>
</tr>
<tr>
<td>SEGMENTED</td>
<td>5.7</td>
<td>6.3</td>
<td>15.0</td>
<td>11.0</td>
<td>5.3</td>
<td>19.3</td>
<td>37.0</td>
<td>26.7</td>
<td>35.7</td>
</tr>
<tr>
<td>EOSINOPHILS</td>
<td>0.3</td>
<td>0.3</td>
<td>2.0</td>
<td>0.3</td>
<td>0.3</td>
<td>0.6</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BASOPHILS</td>
<td></td>
<td></td>
<td>0.3</td>
<td></td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LYMPHOCYTES</td>
<td>12.0</td>
<td>4.0</td>
<td>11.7</td>
<td>15.0</td>
<td>1.3</td>
<td>13.3</td>
<td>2.0</td>
<td>11.7</td>
<td>5.7</td>
</tr>
<tr>
<td>PLASMA CELLS</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>MONOCYTES</td>
<td>4.0</td>
<td>1.0</td>
<td>6.0</td>
<td>1.7</td>
<td>0.3</td>
<td>1.0</td>
<td>1.3</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>RETICULUM CELLS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEGAKARYOCYTES</td>
<td></td>
<td>0.3</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RUBRIBLASTS</td>
<td>20.0</td>
<td>8.7</td>
<td>2.0</td>
<td>1.7</td>
<td>6.3</td>
<td>1.6</td>
<td>6.0</td>
<td>5.3</td>
<td>0.7</td>
</tr>
<tr>
<td>PRORUBRICETES</td>
<td>20.0</td>
<td>15.3</td>
<td>3.7</td>
<td>5.3</td>
<td>17.3</td>
<td>3.3</td>
<td>2.7</td>
<td>4.3</td>
<td>0.7</td>
</tr>
<tr>
<td>RUBRICETES</td>
<td>12.7</td>
<td>31.7</td>
<td>17.0</td>
<td>29.0</td>
<td>22.7</td>
<td>18.3</td>
<td>17.3</td>
<td>13.7</td>
<td>20.7</td>
</tr>
<tr>
<td>METARUBRICETES</td>
<td>4.0</td>
<td>18.3</td>
<td>9.7</td>
<td>15.0</td>
<td>13.7</td>
<td>10.0</td>
<td>5.3</td>
<td>12.3</td>
<td>3.3</td>
</tr>
<tr>
<td>MEGALOBASTS</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1.—Photomicrograph of blood film, 3–15–55, before therapy. × 930.

were absent. Microscopic examination of the urine sediment revealed an occasional white blood cell and a moderate number of crystals of an unidentified type.

The stool was formed and of normal consistency. The guaiac test was negative. No ova nor parasites were seen. A single determination of the stool fat revealed 2.9 Gm. of fat per 100 Gm. of dried stool, but we were unable to make a quantitative collection. At the time his diet contained approximately 25 Gm. of fat daily. It was believed that he did not have a defect of fat absorption.

Serum total protein was 5.4 Gm. per cent, of which 3.7 Gm. per cent was albumin and 1.7 Gm. per cent globulin.
X-ray studies of the chest, including fluoroscopy, revealed no abnormalities. Gastrointestinal x-ray study revealed no abnormalities. The small intestinal pattern was normal. The spleen appeared moderately enlarged.

Several relevant studies were not done on this admission, but were obtained later. A gastric analysis was done on August 23, 1956. There was no free hydrochloric acid in the fasting specimen, but 17.5° of free acid appeared after histamine stimulation. A paper electrophoresis study of his hemoglobin on September 20, 1955 was normal. A determination of fetal hemoglobin made on December 6, 1956 was 6.1 per cent. Schilling tests were made on August 21, 1956 with an excretion in the urine of 5.8 per cent of the administered B₁₂, and on December 19, 1956 with an excretion of 4.8 per cent. Following the addition of intrinsic factor he excreted 7.6 per cent on December 26, 1956.

Red cell indexes were not measured until February, 1956. Thirteen determinations were made between then and December 5, 1956, when nucleotide therapy was begun. The mean corpuscular hemoglobin concentration was as low as 27 per cent twice and as high as 32 per cent once, the average being 29.1 per cent. The mean corpuscular volume varied from 80 cu.micra, or less, three times to over 100 cu.micra twice, the average being 91.1 cu.micra. The hemoglobin was low when the lowest readings were obtained and at its best level when the highest readings were obtained, but this relation did not hold for all the determinations. The mean corpuscular hemoglobin varied similarly, the average being 26.4 µg.

Course

Effect of vitamin B₁₂, folic acid and ascorbic acid.—On March 17, 1955 vitamin B₁₂ was begun intramuscularly in a dose of 500 µg. every two days for five doses. Folic acid therapy was also begun with an injection of 15 mg. intramuscularly every three days for three doses. The patient was continued on the previous oral vitamin preparations supplying 50 mg of ascorbic acid, 4 mg. folic acid and 4 µg. vitamin B₁₂ daily. It will be noted in figure 2 that there was a moderate rise in reticulocyte count over the next 16 days, but during the same time there was a progressive fall in the hemoglobin level. There was no appreciable difference in the white blood cell count or the differential. The appearance of the red blood cells on smear remained unchanged. A bone marrow examination on March 26, 1955 still showed changes of the pernicious anemia type (fig. 3). There were now 74 per cent cells of the rubricytic series. It was considered that parenteral vitamin B₁₂ and folic acid were ineffective. He did not receive these vitamins again until March, 1956, when he was given 1,000 µg. of vitamin B₁₂, 15 mg. of folic acid and 100 mg. of ascorbic acid intramuscularly three times a week for 13 doses (fig. 4). At the end of this time the hemoglobin was lower than it had been for some months. Again it will be noted that 1,000 µg. of vitamin B₁₂ given with the Schilling test on August 21, 1956 did not prevent the striking fall in hemoglobin following cessation of prednisone therapy shortly thereafter.

Effect of pyridoxine.—Pyridoxine HCl in a dose of 50 mg. intramuscularly daily was begun on March 24, 1957. During the next eight days there was no discernible effect (fig. 2).

Effect of cortisone and prednisone.—On April 2, 1955 the child was given a transfusion of 100 ml. of whole blood and was permitted to leave the hospital at the insistence of the parents. For want of rational therapy the child
was begun on cortisone 75 mg. daily on April 3, 1955. On a return visit on April 18, there was striking improvement with rise of the red blood cell count to 4.5 million per cu.mm. and of the hemoglobin to 10.5 Gm. per cent. The white blood cell count and differential were normal. The dose of cortisone was gradually reduced. On May 5, 1955 the hemoglobin reached a high point of 11.8 Gm. per cent. It fell after discontinuation of the cortisone, and the drug was re instituted on June 20, 1955. Again there was a rise in the red blood cell count, hemoglobin, white blood cell count and neutrophils, with a mild reticulocytosis. Thereafter, the hemoglobin level averaged about 10 Gm. per cent and the morphologic abnormalities of the erythrocytes and of the marrow persisted. White blood cell count, differential and platelets remained normal. On November 1, 1955 the cortisone was changed to prednisone in the dosage indicated in figure 4. He remained in partial remission until prednisone was discontinued on October 5, 1956. Ten mg. of prednisone daily was usually sufficient for maximal effect, but on April 2, 1956 the hemoglobin had dropped to 8.3 Gm. per cent while on this dose. The hemoglobin rose promptly to the usual level when the dose was increased to 15 mg. daily and did not drop upon subsequent reduction.
Studies on excretion of orotic acid.—A prominent feature of the child's course throughout this period was the passage of crystals in the urine. If he maintained a good urine output the freshly voided urine would not contain crystals. Large amounts would crystallize on standing. When his intake of fluids was reduced, as always happened when he was ill, his urine excretion would be scanty, he would void infrequently, and crystals would precipitate in his bladder. On several occasions he developed urethral obstruction, and on one occasion right ureteral obstruction. Fortunately the obstructions cleared each time after fluids were forced.

We were unable to identify these crystals until the help of one of us (J.A.B.) was solicited. It was then found that the ultraviolet absorption spectra of the crystals in both acid and alkaline media were identical to those of an authentic sample of orotic acid (fig. 5). Paper chromatography with the use of four different solvent systems revealed identical Rf's for the crystals and the authentic orotic acid (table 2). Melting point studies on the crystals and two derivatives of the crystals confirmed the identification (table 3). Analysis of a single sample of the crystals for carbon, hydrogen and nitrogen content revealed only fair correlation between actual and theoretical values. Column chromatography using a Dowex-1-chloride column proved to be a relatively easy and quite reliable method of identifying and measuring orotic acid in the urine. Use of an automatic fraction collector provided excellent resolution (fig. 6), but a hand column system (fig. 7) proved to be considerably faster. The patient was found to excrete orotic acid in amounts as high as 1.5 Gm. per day. We have studied the urines of 73 other people for evidence of orotic acid. These have included four apparently normal children; the parents and three siblings of the patient; 19 people with various types of megaloblastic anemia, some in relapse,
some after treatment, including intestinal malabsorption syndromes, nutritional megaloblastic anemia, the anemia due to primidone and dilantin sodium, as well as Addisonian pernicious anemia; two patients with Di Guglielmo's disease*; 10 patients with no hematologic disease and no anemia; and 33 patients with a variety of anemias. Of all these, none have shown any orotic acid in the urine.

*Urine samples obtained through the courtesy of Dr. Mario Baldini, Boston, Mass.
The ultraviolet absorption spectra of authentic orotic acid compared to the urinary compound. The samples were run in both acid and base as indicated, with the unknown at a slightly lower concentration to prevent confusion of the curves. A Beckman DK-2 recording spectrophotometer was used. The authentic sample was obtained from California Foundation for Biochemical Research, Los Angeles, and is CfP quality.

**Table 2.**—Paper Chromatography Data

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>R_f's</th>
<th>Authentic Orotic</th>
<th>Urinary Compound</th>
<th>Block's Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-butanol:ethanol:formic acid:water, 50:15:10:25</td>
<td>0.41</td>
<td>0.38</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>n-propanol:formic acid:water, 70:10:20</td>
<td>0.39</td>
<td>0.38</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>n-butanol:formic acid:water, 77:10:13</td>
<td>0.25</td>
<td>0.25</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>n-propanol:water, 60:40</td>
<td>0.53</td>
<td>0.53</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>n-propanol:water, 60:40</td>
<td>0.57</td>
<td>0.57</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>isoamyl alcohol (water sat'd):formic acid, 90:10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.21</td>
<td></td>
</tr>
</tbody>
</table>


| California Foundation for Biochemical Research, CfP.

**Effect of uracil.**—Figure 8 shows the failure of uracil given orally in a dose of 2.5 Gm. per day over a 10 day period to effect any change in the reticulocyte count or hemoglobin level. An attempt was made to collect 24 hour urine samples for determination of orotic acid, but at this time we were unable regularly to obtain reliable collections from our patient, now age two. Later we were able to devise a collection system which worked quite satisfactorily, primarily because of the heroic efforts of the patient's mother, to whom we are most grateful. The determinations made during the period of uracil administration which we think most nearly accurate suggest that
TUBE NUMBER

MEGALOBLASTIC ANEMIA AND OROTIC ACID EXCRETION

TABLE 3.—Melting Point Data

<table>
<thead>
<tr>
<th></th>
<th>Authentic Compound</th>
<th>Urinary Compound</th>
<th>Literature Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orotic</td>
<td>318–320d.†</td>
<td>315–317d.</td>
<td>323d.†</td>
</tr>
<tr>
<td>Orotic</td>
<td>338d.†</td>
<td>335d.</td>
<td>345d.†</td>
</tr>
<tr>
<td>5,5-dihromobartituric acid</td>
<td>216–219d. §</td>
<td>217–218d.</td>
<td>236–239d. †</td>
</tr>
<tr>
<td>Methyl ether derivative</td>
<td>298–300d.†</td>
<td>299–301d.</td>
<td>298–300d.</td>
</tr>
</tbody>
</table>

*The first set of values was obtained by putting the sample in a cold bath and raising the temperature at about 4° per minute; the second set by putting the sample in a bath at 330. This procedure corresponds to that of Mitchell and Nyc (J.Am.Chem.Soc. 69:674, 1947) whose values are quoted above.

†Our values are probably lower than the literature values because of the difference in conditions. Note that in the case of orotic per se 20° differences are obtained depending upon the method of procedure in determining the m.p. or more properly, the decomposition point.

§California Foundation for Biochemical Research, CFP, or derived therefrom.

¶Eastman Kodak Company, white label.

Fig. 6.—Column chromatogram of patient’s urine. Chromatograms of this type were obtained by applying a suitably diluted sample of urine adjusted to pH 8.0 containing approximately 150 to 200 optical density units measured at 260 μ to a 1 x 10 cm. Dowex-1-Chloride column. The column was washed with twice the sample volume of water and eluted with HCl using a continuous gradient elution system collecting 5 ml. samples using an automatic fraction collector. The 290 ml. mixing flask contained water at the beginning; 425 ml. 0.015 N HCl, 200 ml. 0.1 N HCl, and 100 ml. 5 N HCl were run successively through the mixing flask from a reservoir under enough air pressure to give a rate of 0.5 to 0.7 ml./min. One hundred ml. of 5 N HCl were then run directly onto the column to remove any uneluted material. Optical density of the elution fractions was read on a Beckman DU spectrophotometer at 260 and 285 μ, only the 260 values being plotted in this figure. The ratio, 285:260, was used for rapid identification of the peaks. Only two of the peaks gave ratios greater than one: That centered at tube #26 is uric acid with a ratio of 2.6 and that centered at tube #109 is orotic acid with a ratio of 1.8.
prior to uracil the daily orotic acid excretion was about 600 mg. and during the last of the period of administration had dropped to about 400 mg.

Effect of prednisolone.—On October 18, 1956, while in relapse following discontinuation of prednisone, he was placed on prednisolone, 20 mg. daily; figure 9 shows the moderate reticulocyte rise and the improvement in hemoglobin which took place. During this period there was a fall in the excretion of orotic acid in the urine, but it still remained at a high level. The white blood cell count and percentage of neutrophils, which had both fallen during the relapse, returned to normal.

Effect of nucleotides.—On December 4, 1956, while he was still in partial remission and receiving 10 mg. prednisolone daily, he was placed on a concentrated yeast extract containing 115 mg. cytidylic acid and 269 mg. uridylic acid per ml.*

Treatment was initiated with 5 ml. daily, and when it became obvious that this was well tolerated, it was increased to 15 ml. daily. Figure 10 shows the reticulocytosis and rise in hemoglobin concentration which ensued. There was a striking reduction in the excretion of orotic acid. A bone marrow examination on December 28, 1956 showed minimal, if any, changes of the megaloblastic type (table 1). The morphologic abnormalities of the erythrocytes also cleared. The hemoglobin concentration rose to a maximum on December 29, 1956 of 14.0 Gm. per cent with a hematocrit of 44 per cent. During the following two months the mean corpuscular volume averaged

*Obtained through the courtesy of Schwartz Laboratories, Inc., Mt. Vernon, N. Y. (UH-CH 5501.)
MEGALOBLASTIC ANEMIA AND OROTIC ACID EXCRETION

Fig. 8.—Effect of uracil on hemoglobin and reticulocytes.

Fig. 9.—Effect of prednisolone on hemoglobin and reticulocytes and on daily excretion of orotic acid and uric acid.

92.5 cu.micra, the mean corpuscular hemoglobin 28.3 µg., and the mean corpuscular hemoglobin concentration 31.2 per cent.

The change in the general condition of the child was even more important. Between July, 1955, and December, 1956, he had gained weight from 23 pounds to 26 pounds. Following the administration of the nucleotides
his weight steadily increased, and by the 22nd day he weighed 29 pounds. He became much more active, began to walk and run, and began to talk more, whereas previously he had seemed retarded in these respects.

Following this response prednisolone was gradually reduced so that the last dose was taken January 1, 1957. There was a slow fall in the hemoglobin, but it did not fall below 11 Gm. per cent until after February 22, 1957, over 50 days later (fig. 11). This is in contrast to the rapid fall following discontinuation of prednisone in October, 1956.

At this point the nucleotides were discontinued. As can be seen in figure 11, the dose had become quite irregular. This was due to an inability of the child to tolerate the material because of diarrhea, which seemed directly related to the dose and was assumed to be due to the high phosphate and salt content. During this period he had a constant chronic otitis media which required myringotomy on several occasions. In addition there was a severe acute upper respiratory infection in mid-January, 1957.

Following discontinuation of nucleotides on February 22 there was a continued drop in hemoglobin and a rise in orotic acid excretion (fig. 11). Arrangements had been made for readmission to the hospital for a trial on cytidylic acid alone, when the child developed varicella. At first this seemed to be mild, but after several days it began to progress, and the child was admitted to the hospital on March 22, 1957. He was in considerable distress and was completely covered with a varicella rash. The mucous membranes were also affected. The red blood cell count was 4,230,000 per cu. mm., the hemoglobin concentration 8.2 Gm. per cent and hematocrit 29
per cent, with MCV 69, MCH 19 and MCHC 27. He developed evidence of pneumonia and heart failure and died on March 25, 1957.

An autopsy showed varicella with massive involvement of the skin, lungs and liver. The pneumonia was sterile and apparently due to varicella alone. The marrow showed a somewhat decreased cellularity with erythroid cells predominating. Some of these were of megaloblastic type. There was no evidence of infiltration of the organs with erythroblastic cells such as would be expected in Di Guglielmo's disease.

**Discussion**

We have not been able to find a report of a case similar to that of this child. There is no mention in the literature of crystalluria occurring in a patient with a megaloblastic anemia. Crystalluria would not have occurred in our patient were it not for his disinclination to take adequate fluids. It was not present when his fluid output was good. Only a systematic study of urine of patients with megaloblastic anemias would reveal the presence of orotic acid, and we have found no report of this having been done.
There are few reports on the effect of the adrenal steroids or ACTH in megaloblastic anemia. ACTH has been administered to several patients with Addisonian pernicious anemia for periods of 8 to 10 days, producing a slight reticulocytosis but no significant hematologic changes.5-7 Two such patients, however, had a partial remission.8 Meyer and Goldfield9 have reported a good response of nontropic sprue to ACTH, and Glass10 has reported a suboptimal response of nontropic sprue to ACTH after failure of response to oral vitamin B12. Spies et al.11 cite two cases of suboptimal improvement of tropic sprue, one to prednisone and one to prednisolone, but indicate that others failed to respond.

Of considerable interest is a more recent report by Doig, Girdwood, Duthie and Knox.12 Prednisolone, 30 mg. daily, was administered for a three week period to nine patients with megaloblastic anemia who were studied with unusual thoroughness. Four had addisonian pernicious anemia, two had a malabsorption syndrome, one had a megaloblastic anemia following partial gastrectomy and two had a megaloblastic anemia associated with rheumatoid arthritis. In these last two cases there was no evidence of malabsorption, nor of intrinsic factor deficiency. Eight of these nine patients responded to prednisolone with a suboptimal reticulocytosis, a slow rise in erythrocytes and hemoglobin and a disappearance of megaloblasts from the marrow. One patient with Addisonian pernicious anemia was given other treatment after one week because of failure to improve.

We have ourselves observed a complete response to ACTH lasting six months in a patient with rheumatoid arthritis and the malabsorption syndrome. There was later an excellent response to vitamin B12. Arrowsmith et al.13 have reported the case of a child diagnosed as having aregenerative anemia limited to the red cell series. Nucleated red cells were severely diminished in the marrow, but after cortisone therapy, which produced a reticulocytosis without rise in hemoglobin, the marrow showed many megaloblasts. Administration of vitamin B12 and folic acid then produced a reticulocytosis and hematologic remission. Subsequent studies after relapse was permitted showed that vitamin B12 and folic acid would not produce a response until cortisone was given. Through the courtesy of Dr. Arrowsmith we have examined the urine of this child and found no orotic acid at a time when the child was doing well hematologically at the age of 5 years.

In the differential diagnosis of our case a number of causes of megaloblastic anemia must be considered. Addisonian pernicious anemia does occur in children and, when it does, free hydrochloric acid may be present in the gastric juice.14 The Schilling test in our patient indicated better absorption of vitamin B12 than is usual in patients with pernicious anemia, although the values were lower than normal. The improvement with the addition of intrinsic factor was equivocal. None of our patients with Addisonian pernicious anemia have shown any excretion of orotic acid in their urine. The failure to respond to parenteral vitamin B12 eliminates this diagnosis.

A nutritional megaloblastic anemia, including the megaloblastic anemia
of infancy, is readily ruled out by the failure of response to a good diet, vitamin $B_{12}$, ascorbic acid and folic acid.

We have no roentgenographic evidence of intestinal abnormalities. Although the stool fat study and vitamin $B_{12}$ absorption studies are somewhat inconclusive, they suggest that there was not a serious absorption defect. The failure to respond to the therapy given parenterally is against the diagnosis of a malabsorption syndrome or intestinal abnormality. We did not find evidence of a fish tape worm during life or at autopsy.

Megaloblasts are sometimes seen in other diseases. Di Guglielmo's disease, or erythremic myelosis, produces anemia with megaloblastic marrow somewhat similar to what our patient exhibited. The course of the disease and the autopsy findings were not those of erythremic myelosis. We have found no report of crystalluria in Di Guglielmo's disease, nor a report of a good response to steroids. We have had the opportunity to test the urine of two such patients for orotic acid and found none. Megaloblasts may be seen in cases of leukemia. Paraf$^{16}$ reviews the literature and reports a case of a 23 year old man who developed a megaloblastic anemia which did not respond to parenteral liver extracts or folic acid or to oral Brewer's yeast. Three months after diagnosis a repeat marrow aspiration revealed transformation to acute leukemia without megaloblasts. The patient died five months later.

Although nearly all reported cases of idiopathic refractory megaloblastic anemia have responded to folic acid or to autolyzed liver,$^{2}$ there are rare cases which have not. Vilter and Mueller$^{3}$ mention a patient followed for 18 months with a megaloblastic anemia of idiopathic origin which failed to respond to vitamin $B_{12}$ given orally and parenterally, to refined and crude liver extract given parenterally, to crude oral liver extract (Valentine's), and to folic acid, xanthopterin, thymine, uracil, methionine and choline. At the time of death the marrow had become almost acellular. E. Roelsen and A. Soeborg-Ohlsen$^{4}$ followed a 40 year old man for nearly four years who had a megaloblastic anemia which was refractory to refined liver extract or vitamin $B_{12}$ parenterally, oral liver extract and yeast, ascorbic acid, folic acid (orally only), paraminobenzoic acid, choline and pyridoxine. Finally he was given ACTH, but died the day following the first dose. This patient had no glossitis or neurologic changes. In neither of these patients is mention made of crystalluria.

The most interesting aspect of our case has been the response to nucleotides. The importance of vitamin $B_{12}$, folic acid and ascorbic acid in pyrimidine and purine metabolism and the disturbed metabolism of nucleic acids in the megaloblastic anemias is well known. We will not attempt to review the voluminous literature, since this has been done very capably by others. Shive$^{16}$ has reviewed the functions of vitamin $B_{12}$ and folic acid in the biosynthesis of purines and pyrimidines. Vilter and Mueller$^{3}$ have reviewed the effects of purines, pyrimidines and nucleotides as well as of vitamin $B_{12}$, folic acid and vitamin C in megaloblastic anemia. A later article by Mueller and Will$^{17}$ attempts to correlate what is known into a unified concept of the
megaloblastic anemias. Welch has reviewed the metabolic role of folic acid. We believe our case throws some light on the site of action of vitamin $B_{12}$ and folic acid in the biosynthesis of the pyrimidine nucleotides.

There is evidence that free purines and the pyrimidines cytosine, uracil, and thymine are not used as such in the synthesis of nucleotides. They are utilized poorly, if at all, in microbiologic systems and by the rat. Nevertheless, certain pyrimidines have been therapeutically effective in some cases of megaloblastic anemia. Uracil in oral doses of 15 to 30 Gm. daily has been effective, and thymine has produced responses in oral doses of 10 to 15 Gm. daily. Vilter and his co-workers have reported two cases of non-Addisonian megaloblastic anemia responsive to folic acid, but not to vitamin $B_{12}$, who exhibited responses to 15 Gm. daily of thymine, after failing to respond to 30 Gm. daily of uracil.

In contrast to these large doses of pyrimidines, Hausmann has reported good responses in two patients with pernicious anemia to a total of 2 Gm. of thymidine given over a ten day period. Others have failed to obtain responses to thymidine in varying doses. Rundles and Brewer obtained a response with thymidine and inosine in one patient. These authors have also produced a suggestive response in one patient with pernicious anemia to the same mixture of uridylic and cytidylic acids which was used in our patient. Two other patients failed to respond. Similarly, one patient failed to respond to intravenous cytidylic acid. Sodium deoxyribonucleic acid produced a suboptimal response in one patient, whereas oral deoxyribonucleic acid was ineffective in another patient.

Current opinion holds that the pyrimidine nucleotides are formed from orotic acid which is transformed first into orotic ribotide and then into uracil ribotide, as illustrated in figure 12. The evidence that orotic acid is in direct line for the formation of uridylic acid and that uracil is not is reviewed by Wu and Wilson. Hammersten et al. have demonstrated formation of thymine deoxyribotide from uracil ribotide, probably through the addition of formate under control of folic acid.

Clinical evidence supporting the role of orotic acid has been supplied by Rundles and Brewer. Orotic acid in oral doses of 3 to 6 Gm. daily was given to a patient with megaloblastic anemia. In three patients, two with pernicious anemia and one with postgastrectomy megaloblastic anemia, the response was good though suboptimal without complete reversal of the marrow. Two of these patients and a third who had had minimal oral treatment for pernicious anemia before beginning orotic acid were maintained on orotic acid for five to seven months, and then relapsed. Two patients had a rise in reticulocytes without other evidence of response, and two others failed to respond at all.

It is to be noted that, although pyrimidine metabolites do not always produce a response in megaloblastic anemias, when they do the effective dose of orotic acid or of the uridylic-cytidylic acid mixture (in our case) is less than the effective dose of thymine, which in turn is less than that of uracil. This is in keeping with the relative utilization of these materials in other
systems and with the postulated pathway of pyrimidine synthesis (fig. 12). From what is known about the metabolic roles of vitamin B₁₂ and of folic acid it is not to be expected that any of these materials would afford universal or complete relief of the metabolic defects resulting from deficiencies of vitamin B₁₂ or of folic acid.

In our patient we postulate a more localized defect, a failure to progress beyond orotic acid, so that this material, excreted in large quantities, was not utilized. Either uridylic acid or cytidylic acid, or both, were utilized. It may be inferred that the difficulty lay either in ribosidation of orotic acid or in the decarboxylation of orotidylic acid to uridylic acid. In either event neither vitamin B₁₂ nor folic acid was able to accomplish this step. It is to be noted that the patient was continuously on folic acid by mouth and presumably had good stores of vitamin B₁₂, having had periodic injections during the period of study.

There is a striking parallel between the behavior of this patient's pyrimidine metabolism as reflected in his orotic acid excretion and in his response to nucleotides and that demonstrated in bacteria by Yates and Pardee. Using pyrimidine-requiring mutants which accumulated carbamylaspartic acid and to a lesser extent dihydro-orotic acid and orotic acid in pyrimidine-free medium, Yates and Pardee demonstrated that this accumulation could be
prevented by the addition of uracil and other pyrimidines to the medium. They were further able to show with the use of enzyme preparations in vitro that cytidine and particularly cytidylic acid were effective inhibitors of the carbamylaspartic acid synthesis and postulated this as the mechanism for feedback control of pyrimidine synthesis in bacteria (fig. 13). It would appear that a similar feedback control mechanism existed in this patient, since the excessive production of orotic acid was inhibited by the administration of the nucleotide mixture. Unfortunately, we were not able to determine the step in the pyrimidine cycle at which the patient’s defect occurred, other than that it must have been after the formation of orotic acid; nor is it possible to say at which step the feedback inhibition took place. It seems probable that similar systems exist both in bacteria and in man.

**Summary**

A case is presented of a child who had a megaloblastic anemia which was not responsive to vitamin B₁₂ nor to folic acid. The erythrocytes were hypochromic and microcytic, but there was no response to iron or to pyridoxine. Neither parent and no siblings had evidence of thalassemia. The autopsy did not reveal evidence of Di Guglielmo’s disease.

A striking feature was the excretion of large amounts of orotic acid in the urine.

Improvement followed administration of adrenal steroid hormones without reversal of the megaloblastic marrow nor striking reduction in orotic acid excretion.

A seemingly complete hematologic remission and a remarkable reduction in orotic acid excretion followed the administration of a crude mixture containing uridylic acid and cytidylic acid while steroid hormones were continued. Before further studies could be made the child died of severe varicella.

**Summario in Interlingua**

Es presentate le caso de un infante qui habeva un anemia megaloblastic non respondente a vitamina B₁₂ o a acido folic. Le erythrocytos esseva hypochromic e microcytic, sed il habeva nulle responsa a ferro o a pyridoxina.
MEGALOBLASTIC ANEMIA AND OROTIC ACID EXCRETION

Le parentes e le fraternos monstrava nulle evidentia de thallassemia. Le necropsia revelava nulle evidentia del morbo de Di Guglielmo.

Notabile esseva le excretion de grande quantitates de acido orotic in le urina.

Melioration sequeva le administration de hormones steroide adrenal, sin le reversion del production megaloblastic del medulla, e sin un marcate reduction in le excretion de acido orotic.

Un apparentemente complete remission hematologic e un marcate reduction in le excretion de acido orotic sequeva le administration de un crude mixtura que contineva acido uridylic e acido cytidylic, durante que le administration de hormones steroide esseva continuata. Ante que altere studios poteva esser executate, le infante moriva de un varicella sever.

REFERENCES
Refractory Megaloblastic Anemia Associated with Excretion of Orotic Acid

CHARLES M. HUGULEY, JR., JAMES A. BAIN, SHIRLEY L. RIVERS and ROBERT B. SCOGGINS

Updated information and services can be found at:
http://www.bloodjournal.org/content/14/6/615.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml