The Familial Aplastic Crisis in Hereditary Spherocytosis. Urocanic Acid and Formiminoglutaric Acid Excretion Studies in a Case with Megaloblastic Arrest

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The etiology of the aplastic or aregenerative crisis in hereditary spherocytosis remains obscure. Patients are not usually seen clinically until the marrow is well in the recovery phase, and if splenectomy is carried out, the crisis is unlikely to recur. Thus the opportunity for carefully studying patients in this phase is rare and the results of the observations are often indecisive.

Studies on folic acid metabolism were made on a girl aged 10 with hereditary spherocytosis who was seen 6 days after the onset of an illness which precipitated an aplastic crisis. Her sister, aged two, was found to have a reticulocytopenia although she remained well clinically.

Case Report

History: R. C., a girl aged 10 years, was well until six days before admission to St. Mary's Hospital. At this time she developed colicky abdominal pain, diarrhea, vomiting, anorexia and fever. On the third day of the illness she was jaundiced with dark urine. Stools remained normal in appearance. On the day of admission the jaundice was less and the urine lighter in color. Her parents had also noticed that she was now very pale.

Past history: The patient was thought to have been jaundiced during the first week of life. At the age of 5½ she had a second episode of jaundice lasting for three weeks, during which her urine was dark, her stools were light and she had profound anorexia. She was not known to have been anemic although she was given oral iron during the first year of life.

Physical examination: The child was extremely pale and slightly jaundiced. She was drowsy with a temperature of 101.4 F. and a pulse rate of 120 minute. There was a harsh systolic ejection murmur, maximal at the left upper sternal edge but radiating widely over the precordium. The liver edge was felt 2 cm. below the costal margin and the spleen was enlarged and firm extending 12 cm. below the costal margin.

Investigations on Admission

Peripheral blood: Six days after the onset of the illness the hemoglobin concentration was 3.9 Gm. per 100 ml. PCV was 12 per cent, and mean corpuscular hemoglobin concentration 32 per cent. One reticulocyte was seen among 4,000 red cells. There were 24,000 WBC per cu. mm. 19,000 being neutrophil polymorphs. The platelet count was 60,000 per cu. mm.

The stained blood film showed well-marked spherocytosis (fig. 1). Nucleated red cells (7 per 100 white cells) were present. Many of these were megaloblasts. Multilobulated neutrophil polymorphonuclear leukocytes were prominent.

Marrow: Sternal marrow aspiration on the seventh day following the onset of
the illness yielded abundant hypercellular fragments. Erythropoiesis predominated. Many of the erythroblasts were megaloblasts; others had a more compact nuclear chromatin pattern (figs. 2 and 3).

Other Investigations

The red cell osmotic fragility was increased, hemolysis in 0.5 per cent NaCl being 28.6 per cent (normal, 0–5 per cent). The direct antiglobulin (Coombs') test was negative. The serum bilirubin was 1.7 mg. per cent of which 1.0 mg. per cent was indirect. Urinary urobilinogen was greatly increased but no bile pigments were present. Serum vitamin B₁₂ concentration, using Lactobacillus leichmanii as the test organism was 770 μg. per ml. Serum thymol turbidity, thymol flocculation, zinc turbidity, alkaline and acid phosphatase, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase were all normal. The total serum protein concentration was 7.1 Gm./100 ml. (albumin 4.2 Gm., globulin 2.9 Gm.). Fecal fat excretion over a consecutive four-day period on a normal diet was 2.8 Gm. per day. Jejunal biopsy using a Crosby capsule showed a normal mucosa. A cholecystogram was normal.

Course and Progress

On admission a liter of blood was transfused and this raised the hemoglobin concentration to 8.3 Gm. per 100 ml. (fig. 4). No further treatment was given. The reticulocyte count, which was virtually zero on day six, rose to 15 per cent on day eight and 38 per cent on day 11. Thereafter the reticulocyte count was maintained between 6 and 12 per cent. The hemoglobin concentration reached 10.2 Gm. per 100 ml. on day 11 and thereafter was maintained in this region. The platelet count rose to 120,000 per cu. mm. on the 10th day and thereafter was maintained in the region of 200,000 per cu. mm. On the 10th day of the illness, the patient became apyrexial and excess urobilinogen disappeared from the urine on the 12th day. On the 20th day the serum bilirubin was 0.8 mg. per 100 ml.
Aplastic Crisis in Hereditary Spherocytosis

A splenectomy was performed by Professor T. Irvine on the 69th day after the onset of the crisis. This was followed by a further decline in the reticulocyte count to less than one per cent and a rise in the hemoglobin concentration to 13.2 Gm. per 100 ml. A bone marrow aspiration was performed at the time of splenectomy and showed normoblastic hemopoiesis (fig. 5).

Family Studies

The paternal grandmother had undergone splenectomy for anemia 30 years ago. Well-marked spherocytosis and increased osmotic fragility were found in the father and a sister aged two.

The patient’s sister, aged two, had a blood count performed on the day following admission of R. C. She was well clinically. The hemoglobin concentration was 9.7 Gm. per 100 ml. and the reticulocyte count was 1.0 per cent. The stained film showed well-marked spherocytosis. The reticulocyte
Fig. 4.—The hematologic changes and urinary excretion of histidine derivatives following 15 Gm. oral doses of histidine hydrochloride in case R. C.

count was zero four days later and rose to 22.9 per cent on day eight, and then declined to 18 per cent on day 10 and 14 per cent on day 12. The hemoglobin concentration remained in the region of 9.6 Gm. per 100 ml. but subsequently rose to 11.0 Gm. per 100 ml. (day 25). The reticulocyte count was maintained between two and four per cent. The red cell osmotic fragility was increased, showing 20.8 per cent hemolysis in 0.5 per cent NaCl.

J. C., aged 32, the father of the two girls, had a hemoglobin concentration between 12.9 and 14.2 Gm. per 100 ml. The reticulocyte count varied from 7 and 11 per cent. The stained blood film showed well-marked spherocytosis and the osmotic fragility was increased, 17.5 per cent of the cells being hemolyzed in 0.5 per cent NaCl.

Urocanic Acid and Formiminoglutamic Acid Excretion Studies

The urinary excretion of these histidine derivatives was studied after oral doses of 15 Gm. histidine hydrochloride. The urine was collected for eight hours into a bottle containing 5 ml. N/HCl. Urocanic acid and formiminoglutamic acid were estimated spectrophotometrically as 5–10 methenyltetra-
Fig. 5.—Sternal marrow of case R. C. taken at the time of splenectomy 69 days after the onset of the illness, showing normoblastic erythropoiesis.

hydrofolic acid by the method of Chanarin and Bennett. Normal adults excrete less than 17 mg. of urocanic acid and formiminoglutamic acid following 15 Gm. of histidine HCl. We have no information concerning this range in children.

The pattern of urocanic acid and formiminoglutamic acid excretion in the patient R. C. is shown in figure 4. Urocanic acid was the predominant histidine derivative present, formiminoglutamic acid being absent or present in small amounts. No attempt was made to identify the unstable compound imidazolone-5-propionic acid which is an intermediary in the conversion of urocanic acid to formiminoglutamic acid.

The excretion of these derivatives on days eight, nine and ten was 216, 620 and 940 mg. respectively. No observation was made on day 11 which was the time of the reticulocyte peak. On days 12 and 14 the excretion was 198 and 197 mg. respectively and this gradually fell to 24 mg. on day 21.

On the two days preceding splenectomy the excretion of urocanic acid and formiminoglutamic acid was 18 and 26 mg. per day respectively. The next observation was made on day 74 (five days after splenectomy) and the excretion had risen to 550 mg. This fell to 345 mg. on day 76, to 99 mg. on day 78 and finally to 21 mg. on day 92 (22 days after splenectomy).

The excretion of histidine derivatives in the father (J. C.) was normal, being 16 and 11 mg. on two occasions. We were unable to persuade M. C., aged two, to swallow the histidine and no observations were made.

DISCUSSION

The events of the aplastic crisis of hereditary spherocytosis have been admirably recorded by Owren, and by Dameshek and Bloom and the pattern in this case is similar. On admission the patient had anemia, thrombocytopenia and reticulocytopenia. However, the hyperactive marrow indicated that re-
covery was underway and this was manifested by the reticulocyte crisis and the prompt rise in hemoglobin. The patient's sister, aged two, also suffering from hereditary spherocytosis, had a temporary reticulocytopenia following the crisis in her sister and must be presumed to have had a similar arrest of hemopoiesis, which, however, was entirely asymptomatic. The precise diagnosis of the febrile episode initiating the crisis is uncertain.

The unusual feature of this case is the megaloblastic character of hemopoiesis in the early stage of the crisis. Megaloblasts were observed in the stained peripheral blood film on admission and this was confirmed on sternal marrow aspiration carried out the next day. However, the marrow was similar to that seen in a patient with severe Addisonian pernicious anaemia 12-24 hours after an injection of vitamin B₁₂. There were many unequivocal megaloblasts, but other erythroblasts had already lost the finely divided chromatin pattern of the megaloblast and this suggested that erythropoiesis was becoming rapidly normoblastic. Megaloblasts have only rarely been observed in the marrow in the aplastic crisis of hereditary spherocytosis. Vaughan found megaloblasts in the peripheral blood films of two patients with hereditary spherocytosis whose crises terminated fatally. In both, the marrow at autopsy was described as having megaloblasts. Megaloblasts were observed in the crisis of hereditary spherocytosis by Shaw and by Greig, Metz, Bradlow, Theron and Morris. In Owen's series, the plates of the marrow of case 4 on the 10-12th day following the onset of the crisis appear to us to be megaloblastic. The cells on day 10 are described as macroblasts. Only marrow obtained 3-4 days before the reticulocyte peak of the recovery phase can be expected to show evidence of megaloblastic change. As in any megaloblastic anemia, recovery from megaloblastic arrest must be associated with prompt reversion to normoblastic hemopoiesis so that marrow in the recovery phase is normoblastic or at best similar to a megaloblastic marrow 12-24 hours after specific antimegaloblastic therapy.

The megaloblastic arrest of hemopoiesis in this case differs from the arrest occasionally supervening in the course of a chronic hemolytic anemia by the acuteness of the onset, by its initiation by a definite febrile episode and by the presence of similar reticulocytopenia in another member of the family.

While megaloblastic arrest may result from any interference with deoxyribonucleic acid synthesis, the massive excretion of histidine derivatives indicates that in this case failure of DNA synthesis was due to interference with the function of the folic acid coenzymes. We have only encountered excretion of histidine derivatives of this order in idiopathic steatorrhea and in acute leukemia. Urocanic acid is almost invariably excreted with formiminoglutamic acid following histidine loading in patients with megaloblastic anaemia (Bennett and Chanarin).

In man and experimental animals, an arrest of hemopoiesis similar in nature to that seen in this patient can be produced by folic acid antagonists. The bone marrow is both hypoplastic and megaloblastic (Thiersch). Our hypothesis is that the events of the crisis in this patient were initiated by a naturally occurring folic-acid antagonist produced in association with the initial febrile illness. The occurrence of severe gastrointestinal symptoms in our patient, as
well as in many of the recorded cases of aplastic crises in hereditary spherocytosis, is reminiscent of the toxic effect of methotrexate and aminopterin. These gastrointestinal symptoms may well be due to arrest of renewal of gastrointestinal mucosa comparable to the arrest of maturation in the marrow. The postulated antagonist would be effective in producing megaloblastic arrest in these patients as opposed to normal subjects, because subclinical folic acid deficiency is usual in chronic hemolytic states (Chanarin, Dacie and Mollin\(^9\)).

Finally, large amounts of urocanic acid and formiminoglutamic acid appeared in the urine following splenectomy. This is not the usual state of affairs following surgery of comparable severity in otherwise normal subjects. It implies that reserves of functioning folic acid coenzymes were virtually absent in this patient and that any additional stress resulted in failure to metabolize single carbon units such as formimino groups. As already pointed out, subclinical folic-acid deficiency is the usual state of affairs in patients with longstanding chronic hemolysis and this may, in part, explain the large increase of histidine derivatives excreted following splenectomy. However, the link between folic-acid antagonists and enzymes involved in folic acid metabolism, such as folic acid reductase, is an extremely stable one and the complex may persist in the tissues for many months (Werkheiser\(^10\)). Thus the effect of the antagonist which we have postulated as initiating the megaloblastic arrest may have persisted well beyond the stage of clinical and hematologic recovery and its presence only demonstrated by the added stress of splenectomy.

**Summary**

A case of hereditary spherocytosis who presented with an aregenerative crisis is reported. Evidence of megaloblastic hemopoiesis was present and was accompanied by the urinary excretion of large amounts of urocanic acid following oral dose of histidine hydrochloride. The severe illness in this patient was accompanied by a reticulocytopenia in a younger sister who, however, remained well clinically.

**Summario in Interlingua**

Es reportate un caso de spherocytosis hereditari in un patiente qui se presentava in un crise aregenerative. Le patiente exhibiva evidentia de hematopoiese megaloblastic associate con excretion urinari de grande quantitates de acido urocanic post le administration oral de un dose de chlorhydrato de histidina. Durante que le morbo de iste patiente esseva sever, un soror plus juvenile de ille habeva reticulocytopenia sed se trovava clinicamente in bon stato de sanitate.

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