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Potentiation of Pteroylglutamic Acid by Ascorbic Acid in Anemia of Scurvy. (20674)

JAMES H. JANDL* AND GEORGE J. GABUZDA, JR.† (Introduced by William B. Castle.)

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and Department of Medicine, Harvard Medical School, Boston, Mass.

The citrovorum factor (CF) is an active metabolite of pteroylglutamic acid (PGA) (1-3). CF replaces PGA in the nutrition of certain microorganisms(4,5) and animals (6,7), reverses aminopterin toxicity(2,6,8), and is effective in the treatment of nutritional macrocytic anemias in man(9-13). The conversion of PGA to CF by rat liver slices(14) or by resting cells of *S. faecalis* A(15) is enhanced by ascorbic acid. Excretion of CF in the urine following PGA injection is increased by ascorbic acid administration in normal man (16,17), whereas in adult human subjects with scurvy the ability to convert PGA to CF is impaired(18). CF is more effective than PGA in treating the megaloblastic anemia occurring in monkeys fed scorbutic diets low in PGA content(19,20). These observations suggest that ascorbic acid deficiency may precipitate certain nutritional anemias by limiting the conversion of PGA to CF. From studies on 2 patients with scurvy and anemia data are here presented which demonstrate a potentiation by ascorbic acid of the effect of PGA upon erythropoiesis.

Methods. Hemoglobin concentration, hematocrit, and red blood cell count were determined every 3 to 4 days by standard technics. Reticulocyte counts were done daily by the dry method(21), and at least 1,000 cells were counted for each determination. Buffy coat ascorbic acid levels were measured by the method of Butler and Cushman(22). The 24-hour urine outputs were collected and stored without preservative in a refrigerator; upon completion of each daily collection, aliquots were taken and stored at −10°C for assay. The PGA and CF activities of these urines were determined by microbiologic assays which utilize the growth response of *L. casei*(23) and of *L. citrovorum*(1,24), respectively. The levels of vit. B_{12} in the serum were assayed by a modification of the method of Ross which utilizes the growth response of *Euglena gracilis*(25). Serum iron concentrations were determined by the method of Kitzes *et al.*(26) employing o-phenanthroline.

Clinical observations. Case 1. A 42-year-old white male, gave an 8 months' history of inadequate food intake, particularly of ascorbic acid containing foods. On admission, he was weak, pale, and poorly nourished. He had a prominent perifollicular petechial rash over the arms, legs, and trunk, and several large ecchymoses on the posterior aspects of both legs. The tourniquet test was positive, but other coagulation studies, including bleeding time, clotting time, and clot retraction, were normal. Ascorbic acid was not present in the buffy coat of the venous blood. Hematologic studies revealed a macrocytic anemia with a hemoglobin of 6.6 g %, a hematocrit of 21.6 vol %, and a red cell count of 2060000/mm³. The bone marrow aspirate contained a hyper-

* Public Health Research Fellow of the National Institute of Arthritis and Metabolic Diseases.

† Welch Fellow in Internal Medicine of the National Research Council.

active immature erythroid series. No megaloblasts were seen. The serum iron level was 137 μg %, with a total iron-binding capacity of 382 μg %. The total serum B_{12} level was 344 $\mu\mu$g/ml, which is within the normal range for this laboratory of 532 $\pm$ 2 standard deviations of 322 $\mu\mu$g/ml.

Case 2. A 49-year-old white male, chronic alcoholic, entered the hospital with complaints of weakness, pallor, soreness of the mouth, and dysphagia. He gave a history of inadequate food intake for about 7 months prior to hospital admission. Physical examination revealed pallor, evidence of weight loss, cheilitis, and a smooth, pale tongue. There was a perifollicular petechial rash and a hyperkeratotic folliculosis over the extensor surfaces of the arms and legs, and over the abdomen. Perineal and scrotal ulcerations were present. The liver was enlarged. The tourniquet test was negative and other coagulation studies, including bleeding time, clotting time, and clot retraction, were normal. Ascorbic acid was absent from the buffy coat of the venous blood. Hematologic studies revealed a white blood cell count of 2100/mm^3, a platelet count of 81000/mm^3, and a macrocytic anemia with a hemoglobin concentration of 2.9 g %, a hematocrit of 9.2 vol %, and a red blood cell count of 710000/mm^3. The bone marrow aspirate contained a predominance of abnormal red and white blood cell precursors, including numerous megaloblasts. Free acid was present in the gastric juice. The serum iron concentration was 169 μg %, with a total iron-binding capacity of 281 μg %. The total serum B_{12} level was 193 $\mu\mu$g/ml. A bromsulfonphthalein retention of 40% was evident 45 minutes after injection, and there was a 4+ cephalin flocculation. X-ray studies of the upper gastro-intestinal tract revealed a hiatus hernia and a nutritional deficiency pattern of the small bowel. The oral glucose tolerance and stool fat contents‡ during a period of controlled fat intake were normal. The patient was transfused with packed cells from 1 liter of blood at the start of the study.

Procedure. Shortly after admission both patients were placed on a diet which consisted of boiled milk, rice, crackers, sugar, coffee, and water, and which thus provided little, if any, ascorbic acid. After an initial control period both patients received daily intramuscular injections of PGA§: 125 and 250 μg for 10 and 12 days, respectively. In addition to the scorbutic diet and PGA, both patients were then given 1 g of ascorbic acid orally daily, for 9 and 10 days, respectively. In both patients, following the administration of ascorbic acid, the signs and symptoms of scurvy cleared and ascorbic acid appeared in the venous buffy coats. During the final period of study, PGA and ascorbic acid were continued in each patient, but a regular diet replaced the scorbutic diet.

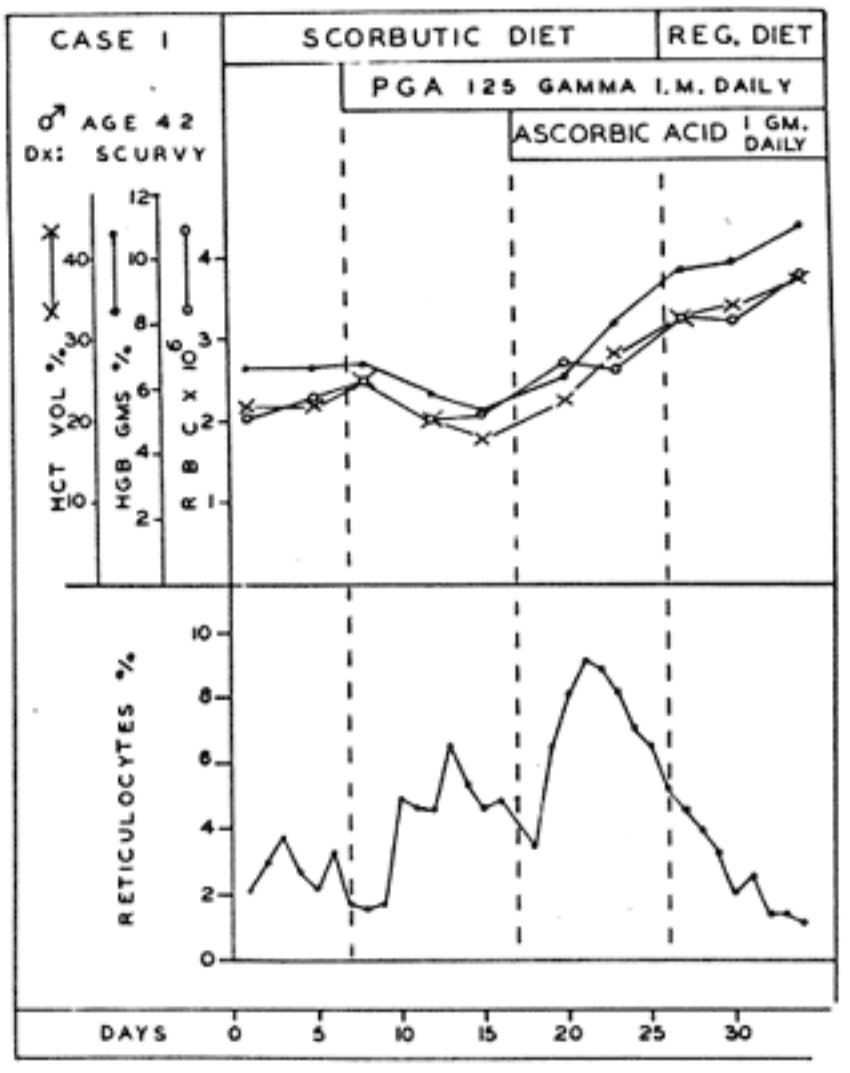

FIG. 1.

Results. When therapy with daily intramuscular PGA was instituted, a reticulocyte response occurred, with a maximum value of 6:5% on the seventh day. (Case 1, Fig. 1.) When ascorbic acid was given in addition to PGA and the scorbutic diet, a second reticulocyte response was observed which reached a maximum of 9% on the fifth day of combined therapy. The introduction of a regular diet produced no further response. The reticulo-

‡ Performed by Dr. Perry J. Culver.

§ "Folvite" Solution, supplied by the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

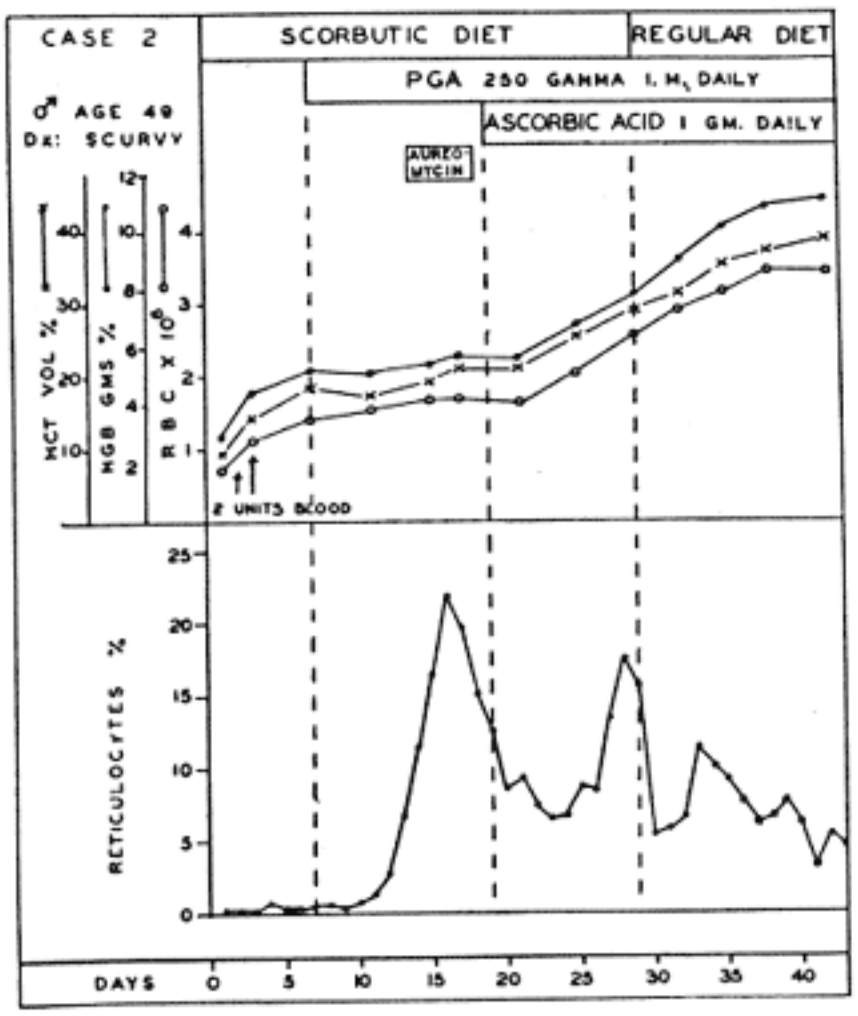

FIG. 2.

cyte responses were followed by increases in hemoglobin concentration, hematocrit, and red blood cell count.

In Case 2, the reticulocyte level was less than 1% during the initial control period (Fig. 2). On the tenth day of daily intramuscular PGA therapy a maximum reticulocyte level of 22% was noted. Three days after this response had begun a 4-day course of aureomycin (1 g p.o. daily) was given for an acute *E. coli* urinary tract infection. With administration of ascorbic acid a second reticulocyte rise occurred with a maximum value of 18% on the tenth day. During the period when the scorbutic diet was replaced by a regular diet with continuation of the PGA and ascorbic acid, a third reticulocyte response developed with a maximum value of 11% on the fifth day. The reticulocyte responses were associated with increases in hemoglobin concentration, hematocrit, and red blood cell count.

Urinary CF activity in Case 1 was immeasurably low throughout the study. In Case 2 urinary folic acid activity (FAA) increased somewhat during PGA administration (Table I). Urinary CF activity did not increase significantly after ascorbic acid therapy.

Discussion. The hematologic responses to the intramuscular administration of small doses of PGA observed in these patients suggest that PGA deficiency was involved in the pathogenesis of the anemia. Whether this PGA deficiency resulted from inadequate intake, from faulty utilization, or from both, cannot be stated with certainty. The secondary reticulocyte responses observed in both patients when ascorbic acid was given in addition to PGA may have resulted from a more efficient conversion of PGA to CF as the scorbutic state was corrected. This interpretation is supported by reports indicating that ascorbic acid facilitates the conversion of PGA to CF by rat liver slices(14), that CF is more effective than PGA in the anemia of scorbutic monkeys(20), and that patients with scurvy given large doses of PGA (10 mg p.o. daily) do not excrete normal quantities of CF in the urine until ascorbic acid is administered(18). The data on urinary excretion of PGA and CF obtained in the present study do not contribute to the interpretation of the hematologic data, since the increases in PGA excretion were small, and since no increase in CF excretion occurred following ascorbic acid administration. This may be related to the small doses of PGA used, to the retention of these substances for correction of tissue depletion, or to an increased requirement for them during active hematopoiesis.

The data presented here do not rule out the possibility that ascorbic acid *per se* might have been responsible for the secondary reticulocyte responses observed. However, previous experience in this laboratory has revealed no evidence of a direct stimulation of erythropoiesis by ascorbic acid alone in anemic scurvy patients. The present observations therefore indicate that ascorbic acid is necessary for optimal erythropoiesis in man through potentiation of the hematologic effect of PGA. This potentiation may be due to the more efficient conversion of PGA to CF which occurs when the scorbutic state is alleviated by ascorbic acid therapy.

Summary. Two men with scurvy and associated anemia, while maintained on a scorbutic diet, displayed hematologic responses to 125 and 250 μg of PGA, respectively, given intramuscularly daily. A potentiation of this

TABLE I. Urinary Folic Acid Activity (FAA) and Citrovorum Factor Activity (CF) in Case 2.

Days of study	Diet	Daily medication: PGA, μg i.m.	Daily medication: Ascorbic acid, g p.o.	Avg daily urinary excretion: FAA, μg	Avg daily urinary excretion: CF, μg
5– 7	Scorbutic	0	0	.505 (.330– .616)	.234 (.123– .323)
8–19	"	250	0	2.747 (.702– 6.280)	.506 (.156–1.036)
20–29	"	250	1	5.105 (1.360–13.524)	.502 (.332– .745)
30–43	Regular	250	1	5.739 (2.520–18.408)	.634 (.413–1.080)

hematologic effect of PGA occurred on subsequent administration of 1 g of ascorbic acid daily in addition to the PGA.

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