

## FURTHER OBSERVATIONS ON THE SPECIFICITY OF THE FOLIC ACID MOLECULE

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IT HAS been established that pteroylglutamic acid (folic acid) stimulates the development of white blood cells, red blood cells and platelets in a variety of animal species and in persons who have certain types of macrocytic anemia. Our studies of folic acid have been concerned chiefly with the striking hematologic response which follows its administration to persons with pernicious anemia, nutritional macrocytic anemia and tropical sprue in relapse. This response has been described in considerable detail,<sup>1</sup> and it has been pointed out that it is indistinguishable from that which follows the administration of refined liver extracts. Nevertheless, the potency of refined liver extracts is out of all proportion to the amount of folic acid they contain, and it is our working hypothesis that the hemopoietic factor in refined liver extracts differs chemically from folic acid per se. The study of the synthetic folic acid molecule offers great promise toward determining something of the nature of blood regeneration. Recently we showed<sup>2</sup> that patients who do not show a hematologic response to methyl folic acid will respond to the folic acid molecule. Since this study was reported, we have investigated the hemopoietic properties of six additional compounds, somewhat related to folic acid in their chemical structure, in persons with Addisonian pernicious anemia, nutritional macrocytic anemia and tropical sprue in relapse. This communication is concerned with these extended observations on the specificity of the folic acid molecule.

### MATERIALS AND METHODS

From a large group of patients, 11 patients with macrocytic anemia were selected for study. Four of these were classified as Addisonian pernicious anemia, 3 as nutritional macrocytic anemia, and 4 as tropical sprue patients. In all cases megaloblastic proliferation and defective maturation, a red blood cell count of less than 2.5 million and a color index of more than 1 were essential diagnostic criteria. For a differential diagnosis of the type of macrocytic anemia, additional criteria were the absence of free hydrochloric acid in the gastric contents even after histamine stimulation in pernicious anemia and the presence of free hydro-

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chloric acid in nutritional macrocytic anemia and sprue. Usually the patients with nutritional macrocytic anemia had diarrhea characterized by loose, dark stools. The diarrhea present in the patients with sprue was characterized by large, liquid to semisolid, foul-smelling stools, varying in color from whitish yellow to yellowish green. A diagnosis of sprue was not made in the absence of steatorrhea. The glucose tolerance curve tended to be flat in both nutritional macrocytic anemia and sprue; and loss of weight, which usually had occurred in both, tended to be greater in sprue than it was in nutritional macrocytic anemia. A history of subsistence on an inadequate diet over a period of years was given by all of the patients with sprue and nutritional macrocytic anemia.

All the patients were admitted to the hospital where thorough medical and dietary histories were obtained and a complete physical examination was made. Rigid dietary control was instituted on admission and continued throughout the duration of the study. Meat, meat products, fish and poultry were excluded. Only 1 pint of milk, 1 hard cooked egg and 3 level teaspoons of butter were allowed daily. Raw vegetables were excluded and all other vegetables were served overcooked. All other foods were allowed in any amount desired.

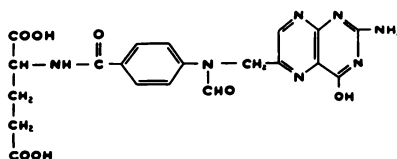


FIG. 1. FORMYL PTEROYL GLUTAMIC ACID (FORMYL FOLIC ACID)

For both the white cell and erythrocyte counts, certified Trenner pipets were used. The hemoglobin content of the blood was determined in grams by means of a Lantz or an Evelyn colorimeter. The reticulocytes were counted in wet preparations by the use of a modified brilliant cresyl blue solution of Dameshek. In all cases permanent fixed preparations of blood smears were made just prior to treatment, and once or twice a week thereafter cell volumes were determined on oxalated venous blood by means of Wintrobe hematocrit tubes. Prior to treatment, bone marrow was obtained and again at the peak of reticulocytosis, and still another specimen was obtained when the reticulocyte count returned to normal. Differential counts were made on preparations stained with both supravital and Wright-Giemsa stains.

After baseline determinations were completed, 20 mg. of the Mg salt of formyl pteroyl glutamic acid (see fig. 1) was administered orally to 1 patient with pernicious anemia and to 1 patient with nutritional macrocytic anemia for ten days. Twenty mg. of the Mg salt of formyl pteroyl glutamic acid (*S. lactis factor*) (see fig. 2) was administered orally to 1 patient with pernicious anemia for ten days. Twenty mg. of N-(4-(4-quinazoline) amino) benzoyl)-glutamic acid (see fig. 3) was given orally to 1 patient with pernicious anemia for ten days. Then the dose was increased to 50 mg. daily for five days. Twenty mg. of pteroyl aspartic acid (see fig. 4) was given orally to 1 patient with tropical sprue for ten days, and 40

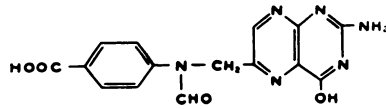


FIG. 2. FORMYL PTERIC ACID (S. LACTIS FACTOR)

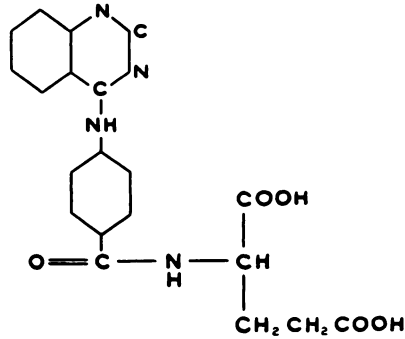


FIG. 3. N-(4-(4-QUINAZOLINE)AMINO)BENZOYL)-GLUTAMIC ACID

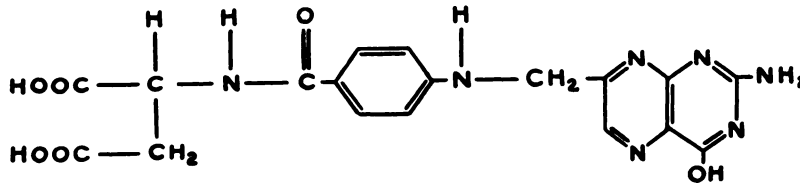


FIG. 4. PTEROYL ASPARTIC ACID

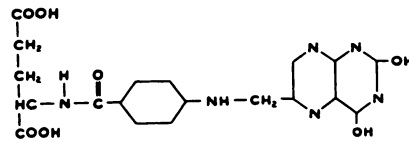


FIG. 5. OXYPTEROYL GLUTAMIC ACID (OXYFOLIC ACID)

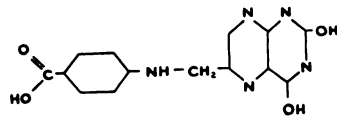


FIG. 6. OXYPTEROIC ACID

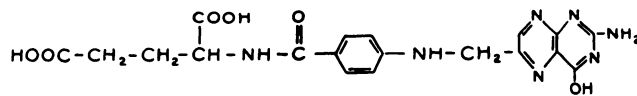


FIG. 7. LIVER L. CASEI FACTOR

N-[4-[(2-amino-4-hydroxy-6-pteridyl)methyl]amino]benzoyl] glutamic acid

mg. was given daily for an additional ten days. Another patient with tropical sprue was given 20 mg. orally for ten days. Twenty mg. of oxyfolic acid (see fig. 5) was

given daily by mouth to one patient with nutritional macrocytic anemia and to 1 patient with pernicious anemia for ten days. Twenty mg. of oxypteroic acid\* (see fig. 6) was given daily by mouth to 2 patients with tropical sprue. If, within ten days, reticulocytosis had not occurred, 10 mg. of folic acid (see fig. 7) was administered until the blood values reached satisfactory levels.

### RESULTS

*Response to Mg Salt of Formyl Pteroyl Glutamic Acid.* Following the administration of this material to the patient with pernicious anemia, the reticulocytes began to rise on the seventh day and reached a peak of 9.6 per cent eleven days after its administration was initiated. This was followed by a slight increase in red blood

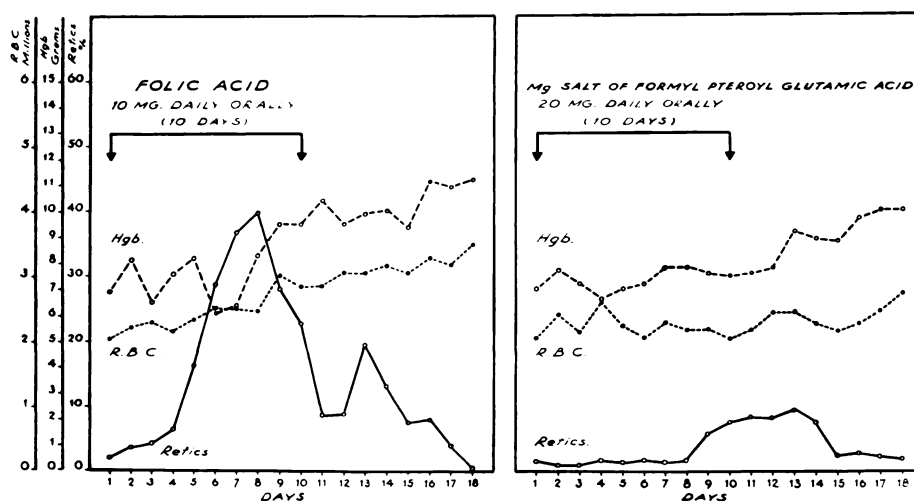


FIG. 8. Comparative response to folic acid and Mg salt of formyl pteroyl glutamic acid in a patient with Addisonian pernicious anemia.

cells, hemoglobin, white blood cells and platelets. The response, however, was poor compared to his response to the administration of folic acid per se during another comparable relapse of the disease when his reticulocytes began to rise on the fourth day of therapy and reached a peak of 39.8 per cent on the eighth day (see fig. 8). By the end of ten days there was an increase in white blood cells and platelets. The response of the patient with nutritional macrocytic anemia to Mg salt of formyl pteroyl glutamic acid was slightly greater, but it was not of the magnitude which followed folic acid per se. Following the administration of Mg salt of formyl pteroyl glutamic acid, the reticulocytes began to rise on the fourth day and reached a peak of 25.4 per cent on the tenth day, whereas on folic acid per se,

\* The Mg salt of formyl pteroyl glutamic acid and the Mg salt of formyl pteroyl glutamic acid were furnished by Dr. Y. SubbaRow of Lederle Laboratories, Inc. The N-(4-(4-quinazoline) amino) benzoyl)-glutamic acid, the pteroyl aspartic acid, the oxyfolic acid and the oxypteroic acid were furnished by Dr. Gustav Martin of The National Drug Company. Only small quantities of these compounds were available so that in no instance did we have an opportunity to test the effect of massive doses.

which was administered during another, but comparable, relapse of the disease, the reticulocytes began to rise on the third day and reached a peak of 60.4 per cent on the eighth day (see fig. 9). There was a slight rise in the red blood cells, hemoglobin, white blood cells and platelets following the administration of the Mg salt of formyl pteroyl glutamic acid but they did not reach satisfactory levels until folic acid therapy was initiated.

*Response to Mg Salt of Formyl Pteroyl Glutamic Acid (S. lactis Factor).* The administration of this material in the dosage given did not produce blood regeneration in a patient with pernicious anemia, whereas an excellent response followed the administration of folic acid per se.

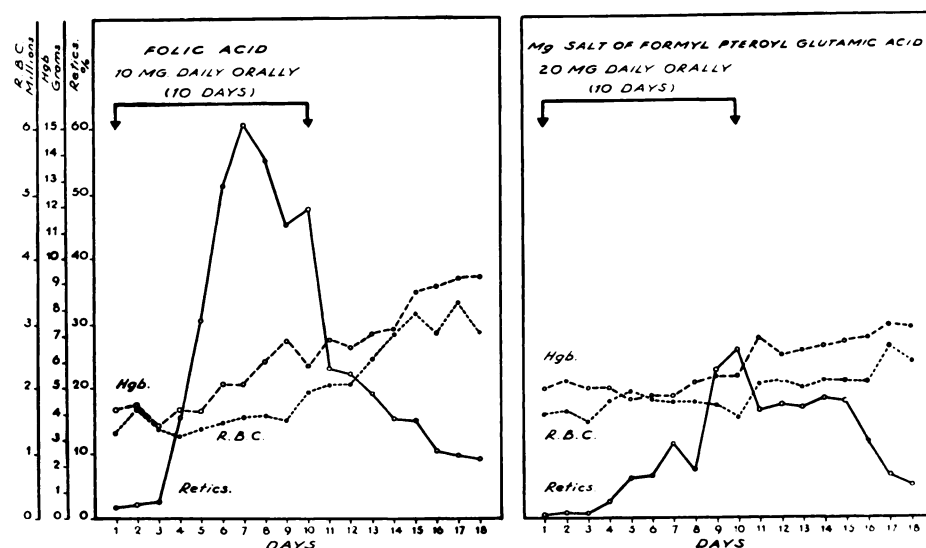


FIG. 9. Comparative response to folic acid and Mg salt of formyl pteroyl glutamic acid in a patient with nutritional macrocytic anemia.

*Response to N-(4-(4-Quinazoline amino) benzoyl)-Glutamic Acid.* Neither the patient with pernicious anemia nor the one with nutritional macrocytic anemia had any hematologic response to this material at the dosage level used. On subsequent therapy with folic acid an excellent response was observed.

*Response to Pteroyl Aspartic Acid.* Blood regeneration did not follow the administration of this material at the dosage level given in either of the 2 cases of tropical sprue. Both these patients later showed a good response to folic acid.

*Response to Oxyfolic Acid.* The administration of this material produced no blood regeneration in the patient with pernicious anemia or in the one with nutritional macrocytic anemia at the dosage level administered. Subsequent treatment with folic acid was followed by an excellent response.

*Response to Oxypteroyl Acid.* At the dosage level used, blood regeneration did not follow the administration of this material in two cases of tropical sprue, whereas folic acid therapy which was given later produced an excellent response.

## SUMMARY AND CONCLUSIONS

Methyl folic acid,<sup>2</sup> N-(4-(4-quinazoline) amino) benzoyl)-glutamic acid, the Mg salt of formyl pteroyl glutamic acid, the Mg salt of formyl pteroyl glutamic acid, the Mg salt of formyl pteroyl glutamic acid, pteroyl aspartic acid, oxyfolic acid and oxypteroyl acid have been studied as to their effect on blood regeneration in selected cases of Addisonian pernicious anemia, nutritional macrocytic anemia and tropical sprue. In the amounts administered, only the Mg salt of formyl pteroyl glutamic acid was effective in producing reticulocytosis and an increase in red blood cells, hemoglobin, white blood cells and platelets, and it was not as effective per unit of weight as was folic acid per se. Presumably this compound is slowly changed into folic acid in the body. It is of special interest that the Mg salt of formyl pteroyl glutamic acid (Streptococcus lactis factor) was negative in producing hemopoiesis. These observations show the very great specificity of the folic acid molecule.

## REFERENCES

- <sup>1</sup> SPIES, T. D.: Experiences with Folic Acid. Chicago, Year Book Publishers, Inc., 1947.
- <sup>2</sup> —, STONE, R. E., AND BRANDENBURG, R. O.: Observations on the specificity of the folic acid molecule. South. M. J. 40: 618, 1947.