

SOME OBSERVATIONS ON THE EFFECT OF FOLIC ACID ANTAGONISTS ON ACUTE LEUKEMIA AND OTHER FORMS OF INCURABLE CANCER

By SIDNEY FARBER, M.D.

THE PRODUCTION of temporary remissions in the course of acute leukemia in children by the administration of the compound, 4-aminopteroylglutamic acid (aminopterin)^{1,2}—a biologic antagonist to folic acid*—has raised a number of theoretic and practical questions. Confirmation of this finding has been reported from several sources³; temporary remissions equally impressive have been obtained in adults with acute leukemia by Dameshek.⁴

It is the purpose of this paper to summarize briefly the status of our observations† on the action of folic acid antagonists on acute leukemia and other incurable forms of cancer for the interest of those now working with these agents, to state the nature of some of the problems which have arisen, and to indicate some directions of further research.

The demonstration by Lewisohn and his colleagues⁵ of the occurrence of complete regression in about one-third of single spontaneous breast cancers in three different strains of mice treated with fermentation *L. casei* factor, later shown to be pteroyltryglutamic acid (Hutchings et al.⁶) and the subsequent synthesis of this compound by SubbaRow and his co-workers⁷ led to our study of the effect of pteroyltryglutamic acid on incurable cancer in man. Among the patients so treated were 11 children with acute leukemia. The occurrence of what we called an "acceleration phenomenon" in the viscera and bone marrow of these patients and an experience with folic acid deficiency experimentally produced in the rat suggested that it would be worth while to ascertain if this acceleration phenomenon might be employed to advantage in the treatment of acute leukemia in children, either by the use of radiation or nitrogen mustard therapy after pretreatment with folic acid or conjugates of folic acid, or by the immediate use of folic acid inhibitors or

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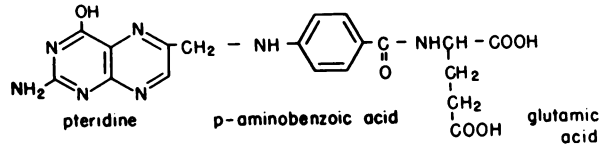
This paper is dedicated to Dr. George R. Minot. It was my privilege when a student to hear his lectures on diseases of the blood. In these he united in masterful fashion the fields of pathology, physiology, and clinical medicine to establish a logical approach to the nature of disease and so to therapy. His announcement, when I was a fourth year student, of the liver treatment of pernicious anemia fired the imagination of all who heard him to a consideration of the role of nutrition in other incurable diseases of unknown etiology. S. F.

* By antagonist to folic acid is meant a substance which possesses the property of inhibiting the growth of *Streptococcus Faecalis R*, or *L. casei* in the presence of marginal levels of folic acid. Reversal of inhibition occurs when the concentration of folic acid in the culture medium is elevated.

† Our studies represent the accomplishment of a group of clinicians and laboratory workers who have joined forces to make possible rapid progress along the lines indicated in this paper. Detailed reports of clinical, experimental, toxicologic and pathologic studies are being prepared for publication.

NUTRITIONALLY ACTIVE SUBSTANCES

**PTEROYL
GLUTAMIC
ACID (PG₂,
Folic Acid)**



**PTEROYL
DIGLUTAMIC
ACID (PG₂,
Diapterin)**

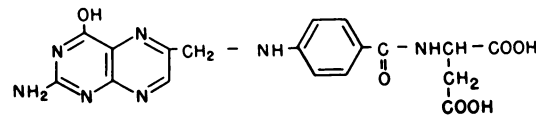
pteridine - p-aminobenzoic acid - two glutamic acids joined by peptide links

**PTEROYL
TRIGLUTAMIC
ACID (PG₃,
Teropterin)**

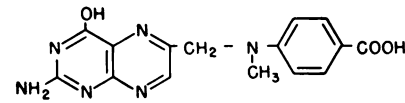
pteridine - p-aminobenzoic acid - three glutamic acids joined by peptide links

BIOLOGICAL ANTAGONISTS

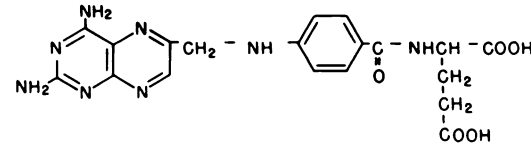
**PTEROYL
ASPARTIC
ACID
(An-Fol A or R)**



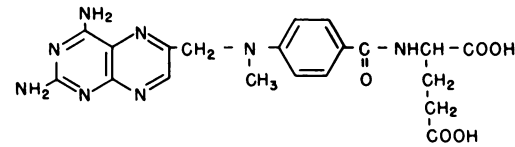
**METHYL
PTEROIC
ACID
(Met-Fol B)**



**4-AMINO
PTEROYL
GLUTAMIC
ACID
(Aminopterin)**



**4-AMINO
METHYL
PTEROYL
GLUTAMIC
ACID
(A-Methopterin)**



**4-AMINO
PTEROYL
ASPARTIC
ACID
(Amino-An-Fol)**

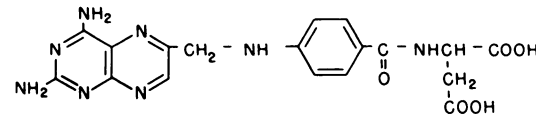


FIG. 1

antagonists.* The first folic acid antagonists—pteroylaspartic acid and methylptericoic acid—were effective enough not only to give the needed encouragement for further research in this direction, but also to prolong the lives of a few children with acute leukemia until more powerful antagonists of folic acid were made available. The first impressive remissions in the course of acute leukemia were produced by the use of aminopterin beginning in November of 1947. These were characterized by a return almost to a normal state in some and to a state almost indistinguishable from normal in others in a group of 10 of 16 children with acute leukemia. The toxicity of aminopterin emphasized the need for less toxic compounds which it was hoped might be even more effective in their carcinolytic action.^{1,2}

Compounds Related to Aminopterin

Observations have been made on children with acute leukemia and on patients with a variety of other forms of incurable cancer treated by two compounds closely related to aminopterin. Both of these were supplied by the late Dr. Y. SubbaRow. These are 4-aminopteroylglutamic acid (amethopterin) and 4-aminoaspartic acid (amino-an-fol).³ A complete account of these studies will be presented elsewhere. In general it may be stated that while amethopterin and amino-an-fol are less toxic than aminopterin, exactly the same toxic changes may be produced when appropriate doses are employed. This holds true for laboratory animals and for man. Remissions in the course of acute leukemia in children equal to those produced by aminopterin may be brought about by the use of amethopterin or amino-an-fol. The effective dose when remissions are obtained in children with acute leukemia lies between 3 to 5 mg. a day for amethopterin, and between 25–50 mg. a day for amino-an-fol, depending upon age, weight, size, and physical condition of the patient. These figures may be compared with a range of 0.5 mg. to 1.0 mg. a day of aminopterin. Because there is some differential in the dose required to produce toxic changes, as compared to the effective dose, it has been possible to shift from one drug to another when early signs of toxicity have become apparent.

Pattern of Therapy

It is impossible to present at this time a pattern of therapy as definite as that governing the use of digitalis, for example, or insulin. Daily white count and physical examination are the best guides to the treatment to be given that day. Too rapid a drop in the white count, diarrhea of unknown origin, the presence of stomatitis, a sore tongue, or ulceration of the mucous membranes of the mouth,

* Acknowledgement is made to the late Dr. Y. SubbaRow and his colleagues in the Research Division of the Lederle Laboratories (American Cyanamid Company) and their associates of the Calco Chemical Division, who are responsible for the chemical research that made possible these studies on children. A particular word of gratitude is expressed not only for the invaluable chemical contributions of Dr. SubbaRow but also for his decision to pursue so effectively by further chemical research the leads which were obtained from these studies on children with acute leukemia. The present plan of study concerning the action of folic acid antagonists is following along the lines decided with Dr. SubbaRow in the spring of 1947. It consists essentially of the study of the action on laboratory animals and on patients with various forms of incurable cancer of related compounds in an attempt to find one which is more effective and less toxic than any we have previously employed.

should serve as reasons for cessation of therapy until the exact cause for these disturbances has been determined. In periods of remissions treatment continues as before, although slightly smaller doses may be administered. In some instances when patients are doing well, intramuscular injection of the compound employed has been given on every other day. Aminopterin apparently is effective also when given by mouth.

Toxicity

Our initial report carried a warning concerning the toxic nature of aminopterin. Stomatitis, ulceration of the mucous membrane of the mouth, smooth tongue, pharyngitis, and atrophic changes in the intestinal epithelium of the type produced by folic acid deficiency in the rat and in the monkey; diarrhea; gastro-intestinal hemorrhage, particularly when there is diffuse leukemic infiltration of the bowel; and depletion of the bone marrow leading to aplasia are the most important changes. Despite efforts to prevent or to overcome quickly the toxic manifestations by the use of liver extract, various vitamin B preparations and folic acid itself in doses up to 200 mg. a day for several days, the most effective treatment appears to be suspension of administration of aminopterin for four to seven days at the first sign of stomatitis or diarrhea of unexplained origin.

The occurrence of hypersegmented polymorphonuclear leukocytes and the presence in the bone marrow of megaloblasts have been observed as important evidences of the effect of the antagonist. It is impossible to state at this time with certainty whether all of the changes produced in acute leukemia by antagonists to folic acids are manifestations simply of a folic acid deficiency. It does appear that the alterations are at the same time more profound and more subtle than those produced by folic acid deficiency alone and that interference with biochemical systems more important than simple competitive substitution of the antagonist for folic acid within cells must obtain. Evidence bearing on this point is being collected.

Hemorrhage

Hemorrhage into the gastro-intestinal tract, the skin, and the genito-urinary tract and the cranial vault, either massive or oozing in character, has always been one of the most serious complications of acute leukemia and one of the important causes of death. Studies now being conducted by our group following the work of Allen and Jacobsen⁹ show that in many children with acute leukemia the level of heparin-like substances in the blood is definitely higher than the normal. While bleeding occurs usually when the level of blood platelets is low, thrombocytopenia may be present without any evidence of bleeding for many months. The longer survival of patients with acute leukemia made possible by folic acid antagonist therapy has brought the problem of hemorrhage into great prominence. The combination of leukemic infiltration of the intestinal tract and toxic effects produced by aminopterin, amethopterin, and amino-an-fol makes for the ready occurrence of gastro-intestinal hemorrhage. Although the exact explanation is not clear it appears certain that hemorrhage occurs more readily if the bone marrow is markedly depressed by the compound employed. The effect may be similar to that pro-

duced in aplastic anemia where gastro-intestinal hemorrhage is a common and serious occurrence. If toxic levels of the folic acid antagonist are employed long enough, the bone marrow may be depressed enough to accentuate the hemorrhagic tendency in leukemia, or to act as the sole cause of the hemorrhage.

Nature of Leukemia

Observations on a girl (M. D.), $8\frac{5}{12}$ years old at the time of her death, and similar experiences with other children have raised a question concerning present conceptions of leukemia. This child lived for twenty-two months after the onset of acute leukemia. Treatment with pteroylaspartic and methylptericoic acid was followed by repeated temporary periods of improvement. She died following uncontrollable oozing from the mucous membranes. Postmortem examination revealed leukemic cells so few in number, in scattered areas throughout the body that the diagnosis of acute leukemia would have been made with hesitation on the basis of that evidence alone. It seems probable that hemorrhage in acute leukemia may be produced by a number of different factors apart from the effect of leukemic infiltrates on the bone marrow and viscera and the thrombocytopenia. The hypothesis seems warranted, that a serious disturbance in the hematopoietic system, or a series of deficiencies in the body responsible for oozing or for massive hemorrhage might still be present in the patient with acute leukemia if every leukemic cell in the body could be destroyed. Acute leukemia, therefore, may be a form of cancer complicated by specific deficiency states—a suggestion that has definite implications for further research.

Types of Leukemia

In the majority of the children with acute leukemia treated it was impossible to diagnose with certainty the exact morphologic type of leukemia because of the primitive nature of the blasts. It would seem logical, and certainly highly desirable to replace or to supplement the morphologic classification of leukemia by one based upon response to specific stimuli, such as the folic acid antagonists. Study of those patients with acute leukemia who failed to respond to these compounds might yield data of value concerning the nature of the disease. A worthy goal is the characterization of the various types of acute leukemia in terms of precise intracellular biochemical deficiencies or alterations.

RESULTS

In a group of approximately 60 children with acute leukemia treated for three weeks or longer with either aminopterin, amethopterin, or amino-an-fol, somewhat more than 50 per cent showed improvement clinically, hematologically of important degree attributable to the action of these compounds. Detailed tabulations of our entire experience with thorough documentation will appear separately. Two of the five children whose case histories were presented in our initial report are still alive (December 21, 1948). Case 1 of that report, a boy of 8, has a history of acute leukemia beginning in February 1947. He was treated first with methylptericoic acid and pteroylaspartic acid. Aminopterin was not given until December

16, 1947. Since then that, or one of the other more powerful folic acid antagonists have been employed. Leukemia is still present and there have been many complications, but he is still alive twenty-three months after the onset of his disease. A second child mentioned in the earlier report, Case 5, has had acute leukemia since August, 1947. He is one of twins and despite his leukemia and almost constant folic acid therapy, he is as tall and as well nourished as his brother. His leukemia, which is still recognizable by studies of bone marrow and peripheral blood, is still under control sixteen months after onset.

The widespread use today of aminopterin in the treatment of acute leukemia has raised for discussion a basis of comparison of results. Any evaluation of treatment of patients with incurable cancer must rest upon a solid foundation of knowledge concerning the life history and biologic behavior of tumors. Acute leukemia, which runs an invariably fatal course, varying from a few weeks usually to six months after onset of symptoms, lends itself readily to comparative studies. Rarely the course may last as long as twelve months, and isolated instances of longer survivals have been observed. The end point of time itself, therefore, should serve as a reliable criterion of the value of any form of therapy.

Spontaneous remissions, either complete or partial, occurred in 10 per cent of 300 children with acute leukemia observed by Dr. Louis K. Diamond,¹⁰ at the Boston Children's Hospital. These averaged slightly less than ten weeks in duration. In two instances a second remission was observed. In almost 75 per cent of these children in whom spontaneous remission was noted, there was a history of infection of important degree immediately preceding the remission. The recent production of remission in acute leukemia by the use of massive blood transfusion makes necessary the evaluation of this factor too, in patients treated with folic acid antagonists. Analysis of our experience permits the statement that the remissions we have described are dependent neither upon infection nor transfusions of blood.

It is obvious that no two children with acute leukemia present strictly comparable problems. Infiltration of the leukemic processes is generalized but there are great variations in the degree and site of involvement. In one, a large subdural accumulation of tumor may alter intracranial pressures to an important degree; in another, the leukemic infiltration in the heart may be responsible for unexpected death. Other variables are the amount of replacement of the bone marrow by leukemic cells, the factors responsible for bleeding, and the occurrence of secondary infections. It should not be surprising, therefore, if one research group reports five consecutive remissions (personal communication from Dr. George Guest, Cincinnati Children's Hospital), or that another group observes a fatal outcome within two weeks after onset of therapy in ten consecutive patients before one remission is observed. The arbitrary limit of three weeks after onset of therapy has been chosen for a basis of comparison. During this period those patients most severely involved will have died or the folic acid antagonists employed will have had an opportunity to effect the tumor infiltrations in the viscera and the bone marrow.

It should be emphasized that all available resources of medicine have been utilized in an attempt to prolong the lives of our patients with acute leukemia. Transfusions, radiation therapy, antibiotics, and specialized dietary measures have all been

employed when indicated. It has been possible, however, to study a sufficient number of patients for long enough periods of time with folic acid therapy alone to permit the accumulation of sufficient data upon which reliable conclusions could be based. It should be expected, therefore, that considerable variation in the results of different investigators will be reported until a sufficiently large experience has been obtained, or until a long enough period has elapsed to permit the use of the period of survival alone as the simplest criterion of therapeutic effect.

The effect of these folic acid antagonists, despite some theoretic considerations which entered into the formulation of early working hypotheses, is not limited to acute leukemia. We have reported¹¹ temporary, definite but inconstant carcinolytic action on patients with apparently unrelated forms of incurable cancer, such as neuroblastoma, and pulmonary metastases from cancer of the bladder, as well as more closely related tumors such as lymphosarcoma and Hodgkin's disease.

The range of carcinolytic action on various types of incurable cancer in man is now being evaluated. The combined action of the folic acid antagonists when employed with other agents used in the treatment of cancer, such as the sex hormones and radiation therapy is under study.

The toxic nature of the compounds employed in these studies and the inconstant and temporary nature of beneficial effects make clear that the value of these compounds is still limited to research. The finding of equally or more effective and less toxic compounds, and an understanding of the reasons for failure in those patients who do not respond are goals which must be reached before more widespread application of the results of these studies is possible.

SUMMARY

A general discussion is presented of the present status of folic acid antagonist therapy in acute leukemia in children and in other forms of incurable cancer. Conclusions reached in our initial report have been supported by a far greater experience. Temporary remissions in acute leukemia as marked as those caused by aminopterin have been produced by the use of two compounds closely related chemically to aminopterin—amethopterin and amino-an-fol, both of which, however, are also toxic compounds. Despite the increasing number of patients in whom temporary remissions have been produced, with survival in some far beyond the usual course of the disease, no evidence has been presented which would justify the use of the word "cure" of acute leukemia. A carcinolytic action on related and on certain unrelated forms of incurable cancer has been observed. Further research for less toxic related compounds with even greater effectiveness is not only justified by these studies but is imperative. The value of this direction of research in cancer has been established.

Two of the most pressing problems demanding solution are concerned with the nature, the prevention, and the treatment of toxic changes, including hemorrhage, produced by these folic acid antagonists and the causes, prevention and mechanism of hemorrhage in acute leukemia. The use of the folic acid antagonists in the treatment of incurable cancer including leukemia must remain in the realm of research until answers to these questions are found.

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