

THE EFFECT OF 4-AMINO-PTEROYLGLUTAMIC ACID (AMINOPTERIN) ON HUMAN LEUKEMIC LEUKOCYTES IN VITRO

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SEEGERS et al.¹ synthesized N-[4-[(2,4-diamino-6-pteridyl)-methyl]-amino]-benzoyl]-glutamic acid, (4-amino-pteroylglutamic acid, or Aminopterin), as a potential antagonist to folic acid, and found it inhibited the growth of *S. faecalis* R. in low concentrations, the inhibition being reversible by means of folic acid. Subsequently, slowing of growth and depression of the red and white blood cell counts were demonstrated in chicks,² weanling rats^{2, 3} and mice.⁴ These effects were produced by doses little short of toxic, and could be counteracted only partially by administration of folic acid. The action on mice of 0.3 parts of Aminopterin per million parts of diet was partially reversible, whereas one part per million invariably caused death within a few days, whatever the quantity of folic acid given.⁴

The fact that a folic acid-deficient diet prevented the growth of Rous sarcoma in baby chicks^{5, 6} led to a trial of folic acid antagonists in an attempt to inhibit the development of this tumor.^{5, 7} Aminopterin was found to be active, but so toxic that only adult birds would tolerate it. Toxicity and tumor inhibition could be neutralized in baby chicks by simultaneous injection of pteroylglutamic, pteroyldiglutamic or pteroyltriglutamic acid. Tumors in successfully treated birds showed, microscopically, "cytological degeneration and, possibly, disruption of mitotic figures."⁸

In human disease, the course of leukemia was found at times to be accelerated by the administration of folic acid.⁹ This suggested that, contrariwise, it might be slowed by folic acid antagonists. Aminopterin, among others, was tried, and in a group of 16 children with acute and subacute leukemia treated with this drug, clinical and hematologic remissions were observed in 10, a rate regarded as greatly in excess of that occurring spontaneously.^{9, 14} Results in adults were less predictable, and toxic effects such as hemorrhage or aplastic anemia were common.^{10, 15} In view of these findings, it has been thought pertinent to study the direct action of Aminopterin on human leukemic cells.

MATERIAL AND METHODS

Leukemic leukocytes were obtained from the peripheral blood of 5 patients with chronic myeloid leukemia. Four of these had previously undergone x-ray treatment at various times, while one was untreated at the time of examination. The latter patient, and one other, were in the terminal phase of their illness, and the blood-picture was dominated by the presence of large numbers of myeloblasts and very early myelocytes.

A total of twelve series of cultures was made by a method, details of which have been published previously.¹¹ In brief, it is a modification of that described by Osgood and Brownlee,¹² and consists in the suspension in 50 per cent human serum or plasma, and incubation, of leukocytes isolated from the peripheral blood. Total, differential and mitotic counts are performed at suitable intervals, and the results

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are charted. In the present series, the culture medium used was fresh homologous serum, and experiments were carried on for twenty-four or forty-eight hours. Aminopterin* was added to cultures at the time of setting them up, unless otherwise stated.

RESULTS

Initially, the concentration of Aminopterin used was 55 γ /ml. (about $1.2 M \times 10^{-4}$), as suggested by Salis.¹³ Identical results were obtained in two experiments. The total and differential counts of treated cultures were found not to differ significantly from those of controls, and stained films showed no evidence of a direct toxic action of Aminopterin on mature or immature cells. There was, however, a complete absence of mitotic figures at all stages of the cultures (fig. 1).

As there were no signs indicating any immediate effects of this drug on resting cells, other than an inhibition of their power to divide, most of the remaining

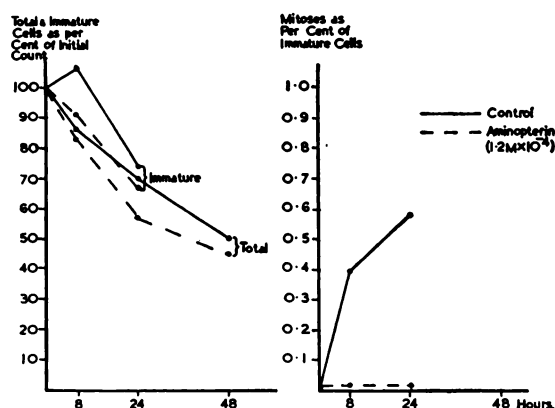


FIG. 1.—Effect of Aminopterin in High Concentration on Total, Differential and Mitotic Counts in Human Leukemic Leukocytes in Vitro.

experiments were confined to an investigation of leukocytes in mitosis. The effects of decreasing concentrations of Aminopterin were next examined. Mitotic inhibition was found to be practically complete in 24-hour cultures with concentrations down to and including $5 M \times 10^{-7}$, while with $5 M \times 10^{-8}$, a 60–90 per cent inhibition was regularly obtained. At lower concentrations, lesser degrees of inhibition were observed (fig. 2). Results showed fairly considerable scattering, owing to the size of the experimental error.

Aminopterin proved equally effective whether added at once or up to eight hours after the cultures were set up (fig. 3). At the latter time, control cultures always showed appreciable numbers of mitoses. Aminopterin was thus capable not only of inhibiting the process of mitosis before its inception in the cultures, but also after it had got fully under way. No abnormal mitoses were seen, but as no counts

* Aminopterin and specially purified folic acid (aldehyde-free) were obtained through the courtesy of Dr. E. Kodicek, Nutritional Laboratory, University of Cambridge, to whom they had been supplied by the Lederle Laboratories Division of the American Cyanamid Company.

were done between eight and twenty-four hours, this fact cannot be taken as conclusive evidence that Aminopterin did not damage cells during division.

Attempts were made to counteract the effects of small quantities of Aminopterin by means of folic acid. Cultures were incubated for one hour in the presence of the

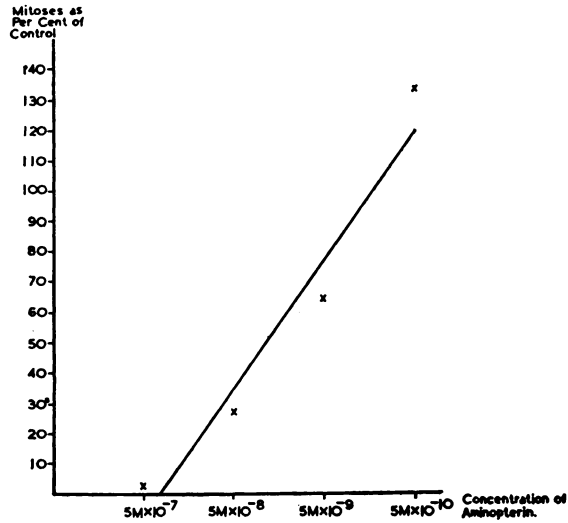


FIG. 2.—Effect of Aminopterin in Decreasing Concentrations on Mitotic Counts at 24 Hours.

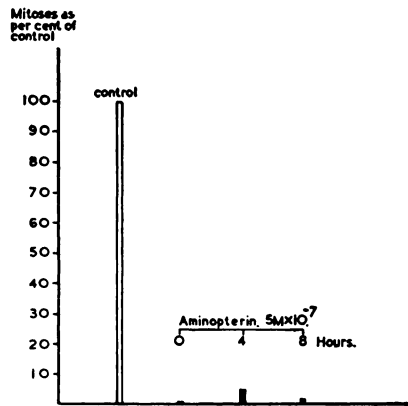


FIG. 3.—Effect of Aminopterin Added at Varying Times on Mitotic Counts at 24 Hours.

latter substance and Aminopterin was then added to them. Two types of response were noted:

(1) In four experiments, concentrations of folic acid of one to ten times that of Aminopterin were apparently inert, but those 100–1000 times stronger produced a partial restoration of mitotic activity (fig. 4). (2) In two others, even high concentrations of folic acid were ineffective in countering the inhibitory action of

Aminopterin (fig. 5, Cases I and II). The second type of response occurred in cultures made from the blood of the two patients in the terminal phase of leukemia. The material under examination was, however, too limited to permit the con-

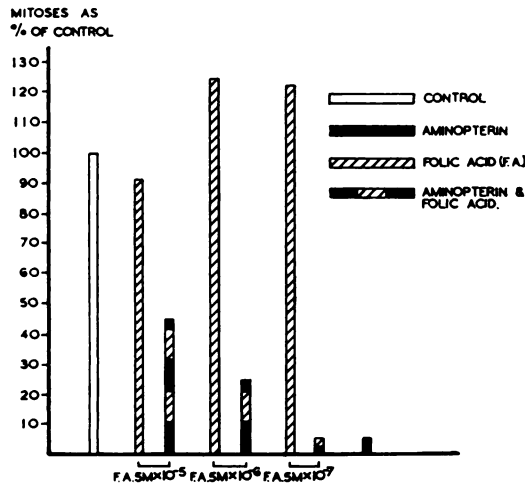


FIG. 4.—Effect of Aminopterin and Folic Acid on Mitotic Counts at 24 Hours (Aminopterin $5M \times 10^{-8}$). Mitosis partially restored by folic acid.

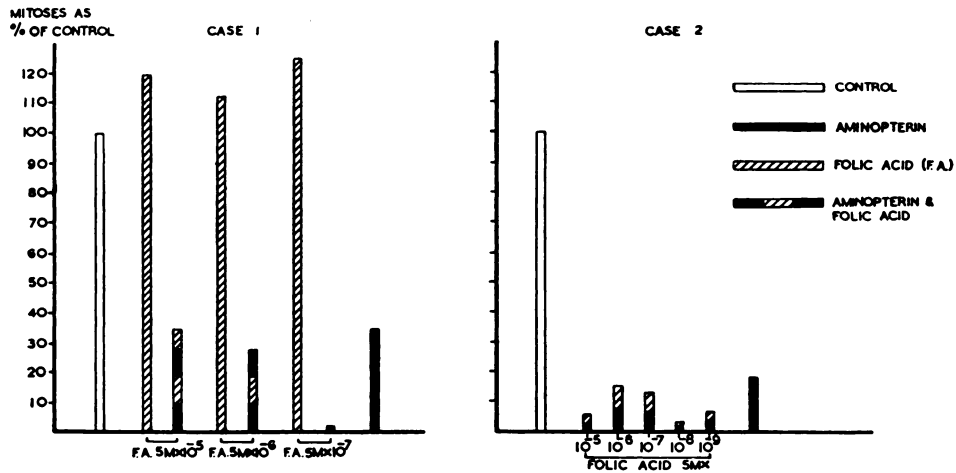


FIG. 5.—Effect of Aminopterin and folic acid on Mitotic Counts at 24 Hours (Aminopterin $5M \times 10^{-8}$). Mitosis not restored by folic acid.

clusion that a fundamental difference between the two groups of cases was thus uncovered.

DISCUSSION

The results presented in this paper show clearly that Aminopterin, acting in vitro, is among the most powerful mitotic inhibitors known, and it is reasonable

to assume that some of the effects noted by others *in vivo*, both in human beings and animals, as the result of the administration of this drug, were also produced by an interference with cell division in susceptible tissues. Attempts to counter the action of Aminopterin by means of folic acid, however, gave ambiguous results. While a partial restoration of mitosis was observed in some cases, no such effects could be achieved in others. Thus, we have little evidence that the inhibition of mitotic activity observed in these experiments was the result of interference with the (presumptive) folic acid metabolism of the leukemic cells. Other observers^{2, 3} have also noticed that folic acid and its conjugates do not appear to inhibit Aminopterin in a strictly competitive way, and that a comparatively small increase of the dose of Aminopterin, beyond the minimum effective one, makes its action irreversible.⁴ The present results raise, but do not answer, the question whether the phenomenon of irreversibility is not due to an action of the drug entirely distinct from any antagonism to folic acid.

A recent paper¹³ reports the occurrence of erythrophagocytosis by normal human myeloid cells *in vitro*, when Aminopterin was added to the cultures. This phenomenon was absent under the rather different experimental conditions here described.

SUMMARY

When added to human leukemic leukocytes *in vitro*, 4-amino-pteroylglutamic acid (Aminopterin) produced a marked inhibition of mitosis in low concentrations. Attempts to counter its action by means of folic acid gave varying results. The mechanism of the observed effect is discussed.

ACKNOWLEDGMENTS

The author wishes to acknowledge gratefully the valuable advice given by Dr. E. Friedmann during the work leading to the preparation of this paper and the financial support provided by the Saltwell Trustees.

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