Prevention of Toxicity of Massive Doses of A-methopterin by Citrovorum Factor.* (18497)

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It has been demonstrated that citrovorum factor (CF) is more effective than pteroylglutamic acid (PGA) in preventing the inhibition of growth of *Leuconostoc citrovorum* by 4-amino-PGA (aminopterin)(1-4). CF has also been shown to prevent the toxic effects of aminopterin in mice(5) and in rats (4), and to be more active on a dry weight basis than PGA in preventing the chemotherapeutic effect of 4-amino-N^{10}-methyl-PGA (A-methopterin) in a strain of mouse leukemia(6,7). Studies by Franklin *et al.* indicate that PGA can prevent the toxicity of aminopterin(8) and A-methopterin(9) only when the antagonists are administered at ranges near the minimum lethal dose. At higher doses, PGA is ineffective. Since earlier investigation has demonstrated that CF is effective at somewhat higher levels of aminopterin(5) than is PGA, it was considered worthwhile to study the effects of CF‡ on the toxicity of high acute and chronic dosage of A-methopterin.‡

Method. Young mice of the Ak stock weighing 17-23 grams and fed the usual diet of Purina Laboratory Chow were used in these experiments. Injections of CF and A-methopterin were given by the intraperitoneal route either once in the acute experiments, or daily for 5 days in the chronic studies. The mice were observed for two weeks after the administration of the first dose of A-methopterin. The results of the studies can be seen in Tables I and II.

Results and discussion. Studies in mice by Ferguson, Thiersch, and Philips(10) have shown the acute LD_{50} for A-methopterin to be 94 mg/kg and the chronic LD_{50} when given daily for 5 doses to be 1.9 mg/kg.

The results in Table I demonstrate that considerable protection against the acute lethal effects of a single dose of 250 mg/kg of A-methopterin may be achieved by as little as one thirtieth that amount of CF given either one hour before or after the dose of antimetabolite. Almost complete protection from this dose of A-methopterin was afforded by doubling the CF to 15 mg/kg. When the CF was given 24 or 48 hours before the A-methopterin, however, no protective effect was noted. There was also little or no significant protection when the CF was administered 4 hours or more after the anti-

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‡ We wish to acknowledge the generosity of the Lederle Laboratories Division of American Cyanamid Company in supplying these compounds.

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TABLE I. Effect of Single Acute Doses of A-Methopterin and Citrovorum Factor.

Dosage in mg/kg A-meth.	CF	CF inj. in relation to A-meth.	Mortality	Day of death 1	2	3	4	5	6	7	8	9
125			10/10			6			4			
250			14/15			5	6		2		1	
250	1.9	15 min. before A-methopterin	7/10			4	2		1			
250	3.8	" " " "	9/10			6	3					
250	7.5	" " " "	5/10			1	1		3			
250	15	" " " "	2/10						2			
250	30	" " " "	1/15						1			
250	30	1 hr " "	3/10				2	1				
250	30	24 " " "	8/10			5	1	2				
250	30	48 " " "	9/10				3		5			1
250	3.8	1 " after "	6/10						6			
250	7.5	1 " " "	5/10			1			4			
250	15	1 " " "	0/10									
250	30	1 " " "	6/14			1			5			
250	30	2 " " "	3/5				2		1			
250	30	4 " " "	3/4			2			1			
250	30	6 " " "	5/5			1	3		1			
250	30	25 " " "	5/5			2	2		1			

TABLE II. Effect of Daily Doses(5) of A-Methopterin and Citrovorum Factor.

Dosage in mg/kg A-meth.	CF	CF inj. in relation to A-meth.	Mortality	Day of death 1	2	3	4	5	6	7	8	9
10	—	—	19/19			6	9	2		2		
10	0.75	15 min. before A-methopterin	1/10								1	
10	1.5	" " " "	0/10									
10	3	" " " "	0/10									
50	—	—	29/29			14	10	2		3		
50	7.5	15 min. before A-methopterin	4/10				1			3		
50	30	1 hr before 1st and 15 min. before all inj. of A-methopterin	0/20									
100	7.5	15 min. before A-methopterin	10/10				1			9		
100	30	1 hr before 1st and 15 min. before all inj. of A-methopterin	0/10									
200	7.5	15 min. before A-methopterin	10/10				2	1		7		
200	30	1 hr before 1st and 15 min. before all inj. of A-methopterin	4/10					2				2
500	7.5	15 min. before A-methopterin	10/10				1			9		

metabolite.

When the CF was given just prior to the A-methopterin (Table II) and a schedule of 5 daily injections was employed, the toxicity of 10 mg/kg (5 x LD_{50}) was almost completely prevented by 0.75 mg/kg of CF. At 50 mg/kg of A-methopterin 7.5 mg/kg of CF, and at 200 mg/kg of A-methopterin, 30 mg/kg of CF protected 60 per cent of the mice.

The clinical effectiveness of simultaneous administration of large doses of CF in *preventing* the toxic mouth ulcerations caused by aminopterin and A-methopterin has been reported(11). The diminished protective action of CF when it was given to mice 4 hours after A-methopterin, however, suggests that CF would not be of great usefulness in *reversing* the lesions of A-methopterin toxicity once they have occurred and the antagonist has been discontinued. Also since it has previously been shown that CF prevents the antileukemic effects of A-methopterin in mice(6) as well as the toxic effects, it is doubtful whether it has any practical usefulness in the therapy of acute leukemia in children.

The fact that the A-methopterin/CF ratio changed relatively little in these chronic studies up to 100 times the LD_{50} of A-methopterin indicates a competitive type of relationship between these two compounds.

11. Schoenbach, E. B., Greenspan, E. M., and Colsky, J., *J.A.M.A.*, 1950, v144, 1558.

This is in marked contrast to the inability of PGA to protect against much smaller doses of A-methopterin(9).

Thus, from these studies and those of others it would appear that aminopterin and A-methopterin are true antagonists of citrovorum factor rather than of folic acid.

Summary. 1. The administration of citrovorum factor 15 minutes prior to seven to thirty times as large a dose of A-methopterin, prevented the toxicity of the antimetabolite at levels ranging from 2 to 100 times the chronic LD_{50}. 2. No significant protective effect was noted when similar dosages of citrovorum factor were given four or more hours after the administration of A-methopterin. 3. A-methopterin appears to be a true antagonist of citrovorum factor rather than of pteroylglutamic acid.

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Role of Aureomycin and Citrovorum Factor in "Folic Acid" Deficiencies.* (18498)

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The use of folic acid antagonists in the treatment of acute leukemia in children has demonstrated that aminopterin and a-methopterin prolong life and give satisfactory clinical improvement in many cases. It has never been adequately explained why some cases similarly managed appear to be entirely unaffected by antagonist therapy. In several laboratory animals the antagonist is toxic to normal bone marrow and produces a leukopenia, growth retardation and hemorrhagic phenomena. No single metabolic action has as yet been suggested to account for these multiple findings. The consideration that more than pteroylglutamic acid (PGA) is antagonized by aminopterin finds some support in the inability of relatively large amounts of this vitamin to overcome small dosages of aminopterin. Our experiments to elucidate the mechanism of action of antifolic acid compounds were designed to test the possibility that more than PGA alone is affected by the antagonists. To obtain information on other factors that may influence the production of a PGA deficiency, it seemed necessary to compare the deficiencies induced by different means. At least three experimental approaches are available to obtain "folic acid" deficiencies: (1) The omission of PGA from a complete diet, (2) The use of PGA antagonists, and (3) The addition of relatively insoluble sulfonamide drugs to the diet. Since the deficiency produced by mere omission of the vitamin from the diet is time consuming and variable, this method was not used. The experiments described below provide evidence that the latter 2 methods may induce other deficiencies in addition to the now recognized PGA deficiency. Accordingly, the term "folic acid" deficiency will be used in the body of the paper to denote the condition observed on the sulfa diet or on the diet containing the antagonist. Since these diets probably produce a multiple rather than a single deficiency, reports by previous workers should be re-evaluated.

Experimental. Sprague-Dawley rats, 24 days of age, were given a synthetic diet consisting of sucrose, 73 parts; vitamin-free casein, 18 parts; salt mixture,† 4 parts; thiamine, 0.5 mg; riboflavin, 0.5 mg; niacin, 1 mg; calcium pantothenate, 2 mg; pyridoxine, 0.5 mg; inositol, 100 mg; biotin, 0.002 mg; and choline, 300 mg; vitamin B_{12}, .010 mg. Two drops of oleum percomorphum was given by mouth twice weekly to each rat as a

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