Prevention of Bone-Marrow Aplasia Induced by Aminopterin with Folacin and Folinic Acid. (21058)

E. C. De Renzo and F. I. Dessau. (Introduced by J. J. Oleson.)

From the Chemical and Biological Research Section, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

The prevention of aminopterin toxicity in the rat, chick, mouse and bacteria has been extensively studied(1-3). As judged by blood picture, weight responses and survival, there appears to be general agreement that folinic acid is far more effective than folacin in overcoming the toxic effects of aminopterin in rats and mice.

During the course of studies on chemically induced bone marrow aplasia, the marked inhibitory effect of aminopterin on rat bone marrow(4,5) was confirmed. This paper presents data showing the effects of the simultaneous administration of aminopterin and folacin type compounds on the cellular elements of rat bone marrow. The procedure followed offers a convenient method for assay of compounds effective against aminopterin. Under these conditions the effects of several pterin compounds of variously reported biological activity have been recorded.

Experimental. Weanling albino rats, reared in our own laboratory were given a diet of the following composition: Labco casein, 18; Cerelose, 73; Wesson Oil, 3; cod liver oil, 2. The following amounts of B vitamins were given as an oral supplement daily: 100 μg thiamine mononitrate, pyridoxin • HCl and nicotinamide, 200 μg Ca pantothenate and riboflavin and 10 mg choline chloride. Ten μg aminopterin was given orally in 0.05 ml or 0.1 ml volume. Immediately after administration of the inhibitor the compound under study was administered as an intramuscular injection. At the end of 5 days surviving animals were killed and femur bone marrow removed and sectioned as described below. The principle upon which the evaluation of bone marrow was based was the degree of injury to development of hematopoietic elements. The criteria for scoring are summarized in Table I.

TABLE I. Evaluation of Injury to Bone Marrow.*

Description	Degree of injury	Score
Normal cellularity	0	0
Foci of diminished cellularity between areas of apparently normal hematopoietic tissue	+	1
Marked general diminution of cellularity; yet all hematopoietic elements present	++	2
Far-going destruction with islets of preserved hematopoietic elements	+++	3
Complete absence of hematopoietic elements	++++	4

Intermediary indices:
0/+ = ½, +/++ = 1½, ++/+++ = 2½, +++/++++ = 3½.

* Sections through the largest plane of pencil of femoral bone marrow. Fixative: 10% formaldehyde, 90% ethanol. Stain: hematoxylin-eosin. Magnification, 200 ×.

Results. The administration of 10 μg aminopterin to weanling rats for 5 days resulted in a score of about 3.0. The effects of simultaneous injection of folacin or folinic acid* (leucovorin) are shown in Table II. That leucovorin was much more effective in preventing the toxic manifestations due to aminopterin is clearly shown. Under the acute conditions of these experiments, the administration of 5 mg folacin was not as effective as 150 μg leucovorin in preventing the toxic effects of 10 μg aminopterin.

The effects of 5 mg pteroyltriglutamic acid, 1 mg N-10-nitroso pteroylglutamic acid, and 1 mg N-10-formyl pteroylglutamic acid were also studied. The data show no evidence of preventive activity.

It was also of interest to investigate the influence of ascorbic acid and/or vit. B_{12} on the response to sub-optimal amounts of folacin. Under the conditions of these experiments, no influence by these 2 compounds

* Folinic acid administered as synthetic Ca Leucovorin.

TABLE II. Prevention of Aminopterin Induced Bone-Marrow Aplasia by Leucovorin and Folacin.

Compound administered*	No. rats	Score‡
None†	94	2.9
Folacin, 20 μg-1 mg	67	2.7
2 mg	24	1.9
3	15	1.3
4	5	1.1
5	15	.5
Leucovorin, 10 μg	20	2.1
20	10	.7
50	10	.3
100	43	.3
125	20	.2
150	5	.0

* All rats received 10 μg aminopterin/day orally. Folic acid and leucovorin administered intramuse.
† These rats were untreated controls, concurrent with those in the table.
‡ Avg values.

when administered alone or in combination with folic acid could be demonstrated.

Discussion. The data presented indicate that leucovorin is far more effective than folacin in preventing toxic effects of aminopterin in the rat. Thus, the evidence from studies on bone marrow is in qualitative agreement with that obtained when growth and survival are utilized as standards of comparison. Sauberlich has reported that leucovorin appeared to be 10 times more effective than folacin when fed in the diet(3). In experiments with mice, Broquist and coworkers (2) have reported that an amount of leucovorin equal to that of administered aminopterin was required to reverse the toxicity of aminopterin when the 2 substances were injected simultaneously. Under the conditions reported in this paper leucovorin appears to be about 50 times more active than folacin in preventing hematopoietic changes in the marrow. Furthermore, approximately 150 μg leucovorin had to be administered to prevent the toxicity of 10 μg aminopterin.

The failure of pteroyl tri-glutamic acid to prevent the aminopterin toxicity even at a level of 5 mg per rat per day is interesting. The tri-glutamate has been reported to replace folacin for chick growth(6) and also to produce hemopoietic responses in pernicious anemia and other megaloblastic anemias(7-9). It is possible that an even larger dose than 5 mg is required to prevent the acute aminopterin effect. This is now under investigation. On the other hand, in view of the lack of effect of a comparable amount of folacin, it is not surprising that the N-10-formyl and N-10-nitroso derivatives, both of which have been reported to possess folacin activity in the chick(6), were without effect under the conditions of these experiments.

Various reports have indicated that ascorbic acid may be involved in the conversion of folacin to folinic acid(10-12). In the present study, however, ascorbic acid administration neither prevented the toxic effects of aminopterin nor influenced the response obtained with folacin. The simultaneous administration (orally or intramuscularly) of ascorbic acid and/or vit. B_{12} with sub-maximum (Table I) amounts of folacin was without effect.

Summary and conclusions. 1. The daily oral administration of 10 μg aminopterin to weanling rats induced a severe aplasia in the bone marrow within 5 days. 2. The simultaneous injection of 150 μg leucovorin completely prevented the aminopterin toxicity. 3. Simultaneous injection of folacin was also effective in preventing toxic effects of aminopterin. Folacin was about 1/50th as active as leucovorin. 4. Ascorbic acid and/or vit. B_{12} did not influence the effect of folacin, *i.e.*, no augmentation of submaximum amounts of folacin by either ascorbic acid and/or vit. B_{12} was observed.

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Effect of Muscular Exercise on Circulating Thyroid Hormone.* (21059)

JOYCE COHEN LASHOF,† PHILIP K. BONDY, KENNETH STERLING, AND EVELYN B. MAN.
From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

Recent studies by many investigators have dealt with the effect of various injurious agents and stimuli on the uptake of I^{131} by the thyroid gland. For example, Bogoroch and Timiras(1) found a consistent decrease in the uptake of I^{131} by the thyroid glands of rats after the injection of formalin or transection of the spinal cord. They also studied the effect of forced muscular exercise on the uptake and found that in one group there was no significant change, while in a second group there was a significant depression. Increased secretion of thyroid hormone on exposure to cold has been demonstrated in rats by Dempsey and Astwood(2) and increased peripheral utilization of the hormone in rats by Rand(3) and by Bondy(4), and in man by Ingbar and Kleeman(5). Bondy also found in rats that 2 hours of swimming caused a pronounced fall in serum protein bound iodine.

The present study was undertaken to determine the effect of muscular exercise in man on the level of circulating hormone and on the rate of disappearance of injected l-thyroxine labeled with radioactive iodine.

Procedure. 1) *Moderate exercise.* One female and 5 male medical students and physicians in good health varying in age from 20 to 36 years walked 14 miles in 4 to 4½ hours. In the first 2 subjects the serum butanol extractable iodine (BEI) concentration was determined at the start, after 7 miles, at the end of the walk, and 5 hours and 16 hours after the end of the walk. Control BEI determinations were made on the same subjects at the same hours during an average day. The second 2 subjects received an injection of 40 μc of radio-thyroxine 5 days before the walk. Serum radioactivity was determined at 9 a.m., 12 N., and 5 p.m. daily for 16 days. On the day of exercise the serum radioactivity was estimated and BEI's determined at the start, at the end of 7 miles, at the end of 14 miles and the following day. The third 2 subjects walked the same distance and BEI determinations performed at the beginning and end of the exercise and on the following morning. 2) *Severe brief exercise.* Six healthy male subjects swam at maximum speed for 15 minutes. All were good swimmers but had not swum for several months and were acutely exhausted at the end. Serum protein bound iodines were estimated before and at the end of the swimming.

Methods. 1) Butanol extractable iodine was measured by the method of Man(6). 2) The radio-thyroxine disappearance curves were performed according to the method of Sterling(7), the subjects being given saturated solution of potassium iodide to minimize re-

TABLE I. Serum BEI's of Subjects Walking 14 Miles (γ %).

Subject	B.	L.	S.	J.	H.	T.
Start	6.8	5.9	4.1	4.7	5.0	4.8
Midpoint, 2 hr	6.4	5.3	4.0	5.2		
End	6.5	5.6	4.4	3.5	4.9	5.2
5 hr after exercise	6.9	6.0				
Next a.m.	6.4	5.9	3.3	4.3	5.9	4.7
△ end-start	−.3	−.3	+.3	−1.2	−.1	+.4

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