the cases, were decreased from an average of 5.80 meq/1 in the pre-period to 5.06 meq/1 at the end of the ACTH period. At the same time the P was decreased from an average for the group of 3.03 to 2.79 meq/1. These decreases in serum K and P are interpreted as an indication of glycogen deposition during the ACTH period rather than being due to increased excretion. The magnitude of the decrease in retention of these two elements appeared to be far too small to influence blood composition.

The mucoprotein and the non-specific hyaluronidase inhibitor of the serums of the patients were both found by our colleagues, R.A. Good, V. C. Kelley, and D. Glick, to be increased at the end of the ACTH period by nearly 100% of the control levels. These elevations were transient, however, essentially normal values being found 4 days after withdrawal of the hormone.

Summary. The effects of ACTH on the fasting blood sugar level and glucose tolerance test; on the potassium and inorganic phosphorus content of the serum; on the nitrogen, phosphorus, chloride, sodium and potassium balances; on the urinary excretion of uric acid, creatinine and adrenal corticosteroids and on the blood eosinophil counts were determined in 5 young children with non-Addisonian (familial) hypoglycemia. The type of response to ACTH was similar in all respects to that of the normal adult. However, under the conditions of this experiment, instead of producing a transient state of diabetes mellitus, as it does in the normal subject, the ACTH appeared merely to reverse the hypoglycemic tendency, with return of the fasting blood sugar level and the glucose tolerance curve to normal. While the eosinophil count returned to normal promptly upon withdrawal of ACTH, the blood sugar remained above the threshold for hypoglycemic reactions for at least 10 days without ACTH in the most severe case in the series (Fig. 1). Administration of 18 mg of ACTH in one dose every 48 hours thereafter served to maintain this one-year-old patient in an essentially non-hypoglycemic state for more than three additional weeks. Results of the study suggest that ACTH may prove to be as effective in the control of this non-Addisonian hypoglycemic disorder as insulin is in the control of diabetes mellitus.

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17255. Prevention of Chemotherapeutic Effects of 4-Amino-N^{10}-Methyl-Pteroylglutamic Acid on Mouse Leukemia by Pteroylglutamic Acid.*

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Certain derivatives of pteroylglutamic acid (PGA) which have in common the substitution of an amino group in the 4 position of the pteridine ring have a demonstrable effect against some strains of transplanted mouse leukemia[1-3] and against solid tumors in mice

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† Fellow of The American Cancer Society, recommended by the Committee on Growth of The National Research Council.

1 Burchenal, J. H., Burchenal, J. R., Kushida, M. N., Johnston, S. F., and Williams, B. S., *Cancer*, 1949, **2**, 113.

2 Law, L. W., *J. Nat. Can. Inst.*, in press.

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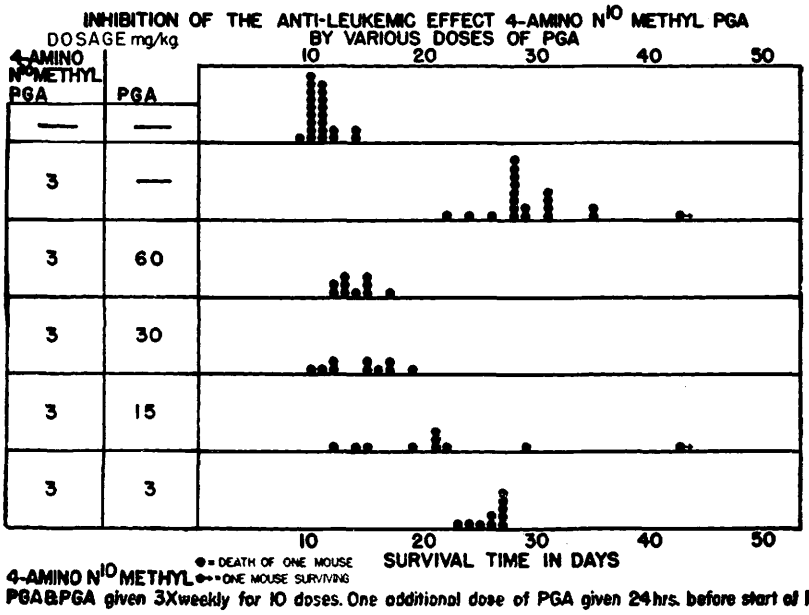

FIG. 1.

and rats.[4-6] The beneficial effects of such compounds in some cases of acute leukemia in children were first reported by Farber *et al.*[7] and have since been confirmed by numerous clinical trials.

In an attempt to elucidate the mode of action of the 4-amino analogs of PGA against leukemic cells, an investigation was undertaken to determine whether prior administration of PGA could prevent the anti-leukemic effects of 4-amino-N^{10}-methyl-PGA.

Experimental. The procedure for inoculating mice of the AKm stock with leukemia Ak 4 was similar to that reported previously.[1,3] 0.1 ml of a saline suspension of leukemic spleen containing 1,000,000 cells was injected intraperitoneally. Forty-eight hours later, treatment with 4-amino-N^{10}-methyl-PGA.‡[8] was started in the maximum tolerated dose of 3 mg/kg 3 times weekly for 10 doses by the intraperitoneal route. Intraperitoneal injections of PGA were started 24 hours after the inoculation of the leukemia and then given one hour before each dose of the antimetabolite. Various dosage levels of PGA were studied against the standard dose of 4-amino-N^{10}-methyl-PGA. As can be seen from Fig. 1 and Table I, PGA administered at levels of 60 and 30 mg/kg by this schedule blocked the anti-leukemic effects of the antagonist. The mice so treated died at approximately the same time as the controls whereas the mice treated with 4-amino-N^{10}-methyl-PGA alone survived at least twice as long as the controls. When the dosage level of PGA was only 15 mg/kg, less blocking of effect was seen and with 3 mg/kg there was almost none.

A less toxic conjugate, pteroyltriglutamic

4 Schoenbach, E. B., Goldin, A., Goldberg, B., and Ortega, L. G., *Cancer*, 1949, **2**, 57.

5 Sugiura, K., Moore, A. D., and Stock, C. Chester, *Cancer*, 1949, **2**, 491.

6 Moore, A. D., Stock, C. Chester, Sugiura, K., and Rhoads, C. P., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 396.

7 Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F., and Wolff, J. A., *New Eng. J. Med.*, 1949, **238**, 787.

‡ We are indebted to Dr. Williams and the late Dr. Subarrow of the Lederle Laboratories for our supply of this compound.

8 Smith, J. M., Jr., Cosulich, D. B., Hultquist, M. D., and Seeger, D. R., *Trans. N. Y. Acad. of Sc.*, 1948, **82**, 10.

TABLE I.
Prevention of the Anti-leukemic Effect of 4-Amino-N^{10}-Methyl-PGA by Pteroylglutamic Acid.

Experiment	Dose, mg/kg		No. of mice	Survival time in days		
	SK 1275	PGA		Range	Mean	S.D.
1	—	—	20	10-18	12	±1.87
	3	60	10	13-24	16	±3.32
	3	—	7	22-41	30	±5.84
2	—	—	18	10-18	13	±1.79
	3	30	20	12-29	17	±1.27
	3	—	19	27-49*	31	±5.3
3	—	—	18	11-17	13	±1.52
	3	60 × 4 30 × 4	17	9-18	16	±2.19
	3	—	11	21-59	30	±9.8
4	—	—	22	9-14	11	±1.22
	3	60	10	12-17	14	±1.51
	3	30	10	10-19	14	±2.86
	3	15	10	12-43†	22	±8.48
	3	3	10	23-28	26	±1.84
	3	—	20	22-43†	30	±4.17

* 2 mice surviving at 49 days.
† 1 mouse surviving at 43 days.

TABLE II.
Prevention of Anti-leukemic Effect of 4-Amino-N^{10}-Methyl-PGA by Pteroyltriglutamic Acid.

Dose, mg/kg		No. of mice	Survival time in days		
4-Amino-N^{10}-methyl-PGA	PTGA		Range	Mean	S.D.
—	—	20	8-13	10	±1.53
—	400	10	9-12	11	±1.38
3	400	9	12-19	16	±2.64
3	200	10	15-20	18	±1.69
3	100	9	13-24	19	±2.82
3	50	10	13-34*	20	±5.49
3	25	10	17-30	25	±3.89
3	12.5	10	19-30	26	±3.29
3	6.25	9	27-34*	29	±2.23
3	—	19	19-34†	28	±3.79

* 1 mouse surviving at 34 days.
† 2 mice surviving at 34 days.

acid (PTGA)[9] was also tested by this technic for its ability to prevent the anti-leukemic effect of 4-amino-N^{10}-methyl-PGA. Table II shows the effect of this conjugate of PGA. Some prevention of the chemotherapeutic action of a standard course of the antagonist was noted at 50 to 400 mg/kg, but none at 6.25 to 25 mg/kg.

Discussion. 4-amino-N^{10}-methyl-PGA[8] was used in preference to 4-amino-PGA in these experiments since its chemotherapeutic effect against leukemia Ak 4 is greater and more consistent.[1,3] The inhibitory action of this anti-metabolite against *Streptococcus fecalis R* has been shown by Franklin *et al.*[10] to be of high degree, but reversible by approximately equal amounts of PGA over a range of 10 to 10,000 γ per 10 ml of medium. In the weanling Wistar rat on a purified diet supplemented with 10.0 mg/kg of PGA, good growth occurred at 1.0 mg/kg of 4-amino-N^{10}-methyl-PGA, but animals fed 3.0 mg of

[9] Boothe, J. H., Mowat, J. H., Hutchings, B. L., Angier, R. B., Waller, C. W., Stokstad, E. L. R., Semb, J., Gassola, A. L., and Subbarow, Y., *Trans. N. Y. Acad. Sc.*, 1948, **10**, 70.

[10] Franklin, A. L., Belt, M., Stokstad, E. L. R., and Jukes, T. H., *J. Biol. Chem.*, 1949, **177**, 621.

the anti-metabolite per kilo of diet all died. When the dosage of PGA was increased to 100 mg/kg the rats at the 3.0 mg/kg level survived, but no protection was noted at the 10 mg/kg level of anti-metabolite. In the chick, the same investigators[10] found a slight inhibition of growth by 3 mg of the antagonist per kilo of purified diet in the presence of 0.1 mg/kg of PGA. When the level of PGA was raised to 10 mg/kg, this inhibition of growth was prevented. Hertz and Tullner[11] found that with this compound inhibition of the estrogen induced response of the chick oviduct could be obtained by 3 daily subcutaneous injections of 0.2 mg each. Blocking of this inhibition was demonstrated with 3 daily doses of 5 mg each of PGA given subcutaneously one hour prior to the injection of the anti-metabolite.

With a closely related antagonist, 4-amino-PGA, the prevention by PGA of toxicity,[12,13] of inhibition of estrogen induced growth of uterus or oviduct,[11] and of the inhibitory effect against mouse leukemia[2] and solid tumors[4] has been observed.

It has been shown above that prevention of the toxic effects of these 2 analogs of PGA may be obtained within narrow ranges at the minimum toxic dose by high dosage of PGA. Since the dose of 4-amino-N^{10}-methyl-PGA used in these experiments was just below the toxic dose, it was to be expected that an effect obtained at this level might be counteracted by PGA. The ratio of PGA to anti-metabolite necessary to prevent this anti-leukemic effect was approximately similar to that required in other studies.[10,11] The ability of PGA and PTGA to prevent the anti-leukemic effects of this anti-metabolite would seem to lend support to the theory that the chemotherapeutic effect of this derivative is due to its action as a metabolic antagonist of PGA.

Summary. The effect of 4-amino-N^{10}-methyl-pteroylglutamic acid in prolonging the survival time of mice with transplanted leukemia Ak 4 can be blocked almost completely by prior administration of 10 to 20 times as much pteroylglutamic acid and, to a lesser degree, by 17 to 125 times as much pteroyltriglutamic acid.

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[11] Hertz, R., and Tullner, W. W., *Endocrinology*, 1949, **44**, 278.

[12] Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 398.

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17256. Effect of Aluminum Hydroxide Gel and Calcium Lactate on Serum Bicarbonate.*

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The observations herein reported indicate that subjects with reduced kidney function respond to the oral administration of suitable amounts of calcium lactate and aluminum hydroxide gel with a marked and sustained elevation of serum bicarbonate. This response occurred both in very young infants with physiologically immature kidneys[1] and in older infants and children with kidney disease. No such increase in serum bicarbonate accompanied the administration of comparable amounts of the two agents to older infants and children with normal kidney function.

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