TABLE I.
Effects of Dibenamine on Blood Pressure, Cardiac Output and Peripheral Blood Flow.

	Blood pressure			Cardiac output, liters/M²			Blood flow, cc/min.		
		After Dibenamine			After Dibenamine			After Dibenamine	
Dog	Control	30 min.	60 min.	Control	30 min.	60 min.	Control	30 min.	60 min.
DF 13	207/175	155/105	135/60	2.5	3.0	4.8	82	45	41
3	150/128	93/75	91/25	1.5	1.45	1.7	4	8	4
14	190/135	198/112	202/140	3.4	4.0	3.8	60	116	135
15	295/242	102/57	Died	6.0	4.7	Died	148	16	Died
16	190/157	95/77	95/70	1.4	1.3	1.3	82	32	12
17	185/162	130/115	122/107	1.2	1.0	1.0	39	36	41
18	205/180	155/130	152/110	1.9	0.9	2	55	28	30
19	220/187	150/87	145/77	1.8	3.9	3.4	48	39	36

increase being the most frequent response. The blood flow during the experimental period, dropped in 16, remained the same in 6 and increased in 2 dogs one of which had a blood pressure rise of 12 mm Hg and the other a fall of 10 mm Hg. The flow in the other 2 dogs, whose pressure was increased, remained constant in one and dropped 30% in the other. The cardiac output dropped in one case only (DF 15), there was an increase in 2 (DF 13, DF 18), and the others did not change significantly.

Dibenamine was administered intra-arterially in 2 to 4 mg/kg amounts in 3 dogs. There was no evidence of any peripheral action.

In 2 dogs epinephrine, 0.01 mg/kg was given at the end of the 30 minutes period, the cardiac output approximately doubled returning to the control level in from 2 to 3 minutes, the heart rate increased between 10 and 15% and the systolic and diastolic pressure fell.

No detectable difference in response was noted in the dog receiving chloralose.

Discussion. Dibenamine in adrenolytic doses produces a significant drop in blood flow to the head or leg, but has little effect on cardiac output of the dog. The drop in blood pressure and the change in the pressure pulse are probably the result of a vasodilation in the splanchnic area which does not include the kidney.[7]

Summary. Following Dibenamine, cardiac output remained essentially unaltered in 5 of 8 dogs, peripheral blood flow decreased significantly in 16 of 24 dogs.

[7] Wang, C., and Nickerson, M., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 92.

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17099. Effect of an Antagonist of Pteroylglutamic Acid on the Leukemia of Ak Mice.*

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In a previous paper[1] hematologic and histologic findings in mice with an induced deficiency of pteroylglutamic acid (PGA) were described, together with a method for producing the deficiency with the aid of a crude chemical antagonist of PGA (so-called "x-methyl PGA," presumably a mixture of 7-methyl- and 9-methyl PGA).†

Following this study we became interested

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[1] Weir, D. R., Heinle, R. W., and Welch, A. D., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 211.

in the influence of this deficiency on the transplanted leukemia of Ak mice. About 75% of this strain develop spontaneous leukemia after the age of 6 months. The intravenous injection of leukemic cells from diseased older animals produces progressive leukemia in 100% of younger animals.[2] It was originally planned to make the transplant after the deficiency, as evidenced by leucopenia, had been established. This approach was found to be unsuitable, probably because the Ak strain is not hardy enough to survive the dietary regime for the preliminary 6 to 8 weeks required for the development of the deficiency, together with the additional time necessary for the growth of the transplanted material. Accordingly, it was decided to transplant the leukemic cells about 15 days after the start of the experiment.

In a preliminary experiment there had been some indication that the parenteral administration of the crude synthetic antagonist of PGA (Lederle N-67A)‡ might be more effective than its oral administration. Accordingly, for comparative study a microbiologically assayed solution of partially purified "x-methyl PGA" was used.‡ The preliminary work also indicated the desirability of pair-feeding the animals in an attempt to rule out the possible effects of inanition as a result of diminished intake of PGA.

Methods. Weanling Ak mice weighing with-

TABLE I. Results with Purified Diet and Added Oral and Parenteral Supplements.

Group	Leukemic animals (see text)	Supplements to purified diet	Survival after transplanting			Leucocytes		"Blasts"	Histological findings		
			Mean days	Max. days	Min. days	Max. daily mean, per cmm	Max. single count, per cmm	Max. daily mean, per cmm	Liver	Spleen	Marrow
1	7	Parent. antagonist	46	71	27	38,000	55,000	1026	++	+++	+++
2	4	Oral antagonist Parent. antagonist Parent. PGA	38	73	22	34,500	55,000	1380	+++	++++	++++
3	4	Oral antagonist Parent. antagonist	53	77	35	14,750	27,000	140	0	+	++
4	4	Oral antagonist Parent. Locke's sol.	45	63	19	17,000	23,000	97	0	0	++
Controls	5	Normal diet No supplement	19	23	16	92,000	108,000	4600	++++	++++	++++

† The crude antagonist was prepared by Dr. M. E. Hultquist and Dr. J. M. Smith, Jr., Calco Chemical Company, by reacting 2,4,5-triamino-6-hydroxy-pyrimidine and p-amino-benzoyl-l(+)-glutamic acid with 2,3-dibromobutyraldehyde in the reaction described by Angier *et al.*, *Science*, 1946, **103**, 667. We are much indebted to Dr. T. H. Jukes of the Lederle Laboratories Division, American Cyanamid Company, who supplied us with this antagonist of PGA.

[2] Weir, D. R., and Heinle, R. W., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 268.

‡ Assayed on *Streptococcus faecalis R*, each ml of the solution used contained the equivalent of 68 mg of a crystalline reference standard supplied by Dr. E. L. R. Stokstad of the Lederle Laboratories. However, it developed subsequently that this microbiologically active fraction is not responsible for the effect of the crude antagonist on animal species.

in 5 g of each other were arranged in 4 groups of 10 animals each and one group of 5 animals (Table I). The composition of the purified diet has been given previously.[1] In this experiment the diet included 1% succinylsulfathiazole from the beginning. All groups were maintained on this diet except the controls. For Groups 2, 3, and 4, 0.5% powdered antagonist was added to the diet. On the 13th day parenteral therapy was started. One cc of the antagonist solution was given daily intraperitoneally to Groups 1, 2, and 3; 20 μg of PGA in 0.2 ml of Locke's solution was given daily subcutaneously to Group 2; and 1 cc of Locke's solution was given daily intraperitoneally to Group 4. On the 16th day all animals were transplanted. The possibility that the potency of the cell suspension might deteriorate during the time of inoculation of a large group of animals was taken into account. Animals from the various groups were taken in rotation so that no one entire group was inoculated either at the beginning or end of the transplanting period.

The animals of groups 2 and 4 were fed the same amount of diet as that consumed on the previous day by the animals of Group 3 (thus, animals No. 11 and No. 31 were fed the amount of food consumed by animal No. 21 on the previous day). Twice weekly all animals were weighed and total and differential leucocyte counts were done. At death, sections of liver, spleen, and bone marrow were prepared for histologic studies.

Results. As in the preliminary experiments it was found that the Ak animals do not survive well under these forms of treatment; many mice died before the leukemia could have developed. Others died during the period when the controls showed florid leukemia, but did not themselves show any evidence of the disease, either by blood counts or by gross or microscopic pathologic examination. Therefore, it was considered justifiable to eliminate from consideration those animals which died early or which did not show any evidence of leukemia at any time. In Group 1, 7 animals remained and in each of Groups 2, 3, and 4, 4 animals remained. All 5 of the controls were dead of leukemia within 23 days after transplanting. Although a reliable demonstration of the effect of the regime on time of survival was prevented by the early mortality of many of the experimental animals, other findings are of considerable interest. The results of the experiment are shown in Table I.

The control animals developed the typical picture of the transplanted disease; all had very high leucocyte counts with blast cells in the peripheral blood, and histologic sections showed extensive leukemic infiltration in the liver, spleen, and marrow. Although all controls were dead within 23 days, the mean survival time of the experimental groups was about 46 days. Control animals with transplanted Ak leukemia almost invariably die within a few days of each other, a circumstance which obtained in this experiment. In our experience the interval between inoculation and death may vary in different experiments, due probably to variation in the donors, but in any one experiment the time of survival of various individuals differs only by a few days. Accordingly, the apparent increase in the mean survival time in the experimental groups was considered to be encouraging in import.

The animals in Group 4 which received the diet and oral antagonist showed the maximal alteration of the leukemic process. The white blood cell counts never rose above the maximal normal of 24,000 per cmm and only small numbers of blast cells appeared in the peripheral blood. Histologically the livers and spleens of these mice showed no leukemic infiltration and the marrows showed only moderate infiltration. Essentially the same picture was seen in Group 3, indicating that the action of the oral antagonist was not significantly altered by the parenteral preparation. This conclusion was supported by the finding that the animals in Group 1, which received the parenteral antagonist without oral antagonist, showed a moderate increase in white blood counts, moderate numbers of blasts, and histologic findings which differed very little from the controls. The animals in Group 2 received the diet with both oral and parenteral antagonist, supplemented with parenterally administered PGA. It had been calculated that a daily dose of 20 μg of PGA would combat all effects of the antagonist. Unfortunately this was not true. However, the

TABLE II.
Results with Normal Mouse Diet and Antagonist Added in Excess.

Group	No. of animals	Diet supplement	Survival after transplanting			Leucocytes		"Blasts" max. daily mean, per cmm
			Mean days	Max. days	Min. days	Max. daily mean, per cmm ×1000	Max. single count, per cmm ×1000	
1	8	No antagonist	16.1	21	14	82	127	1,800
2	8	2% PGA-antagonist	39.0	72	31	45	88	4,200
3	8	4% " "	57.0	74	41	22	32	1,600

dose was sufficient to allow the development of moderately high white counts with moderate numbers of blasts and histological changes which could not be distinguished from the controls.

Within each group the histologic changes were surprisingly uniform in the individual animals. The plus values given in Table I apply to each individual animal and are not means for the group.

In all the experimental groups growth was arrested and there was a steady loss of weight. In this respect none of the groups differed from the others.

Because of the high mortality of the experimental animals on the purified diet, as described in Part I of this experiment, the effect of the PGA-antagonist, when added to an unpurified diet known to be nutritionally adequate for the mouse (Purina dog chow), was studied.

Methods. In preliminary experiments, PGA-antagonist at levels of 0.5% and 1.0% had had some effect on the progress of the leukemia but not enough to be statistically significant. Accordingly, 24 Ak mice, weighing within 5 g of each other, were arranged in 3 groups of 8 animals each and were fed Purina dog chow ground into a coarse powder. Group 1 (controls) was given no supplement, while Group 2 and Group 3 were given the antagonist at levels of 2 and 4%, respectively. The animals of Groups 1 and 2 were fed that amount of diet consumed by Group 3 on the previous day. Twice weekly all animals were weighed and total and differential leucocyte counts were done. On the fifteenth day all animals were transplanted. At autopsy, sections of liver, spleen, and bone marrow were made for histologic study.

Results. In contrast to the experience described in Part I, the dietary regimen of Part II was very well tolerated by all animals. None of the animals died in the preliminary stages and all developed evidence of leukemia before death. The results are given in Table II. With 2% antagonist the mean survival time was more than doubled, and with 4% it was more than tripled. In neither of the supplemented groups did an animal expire until at least 10 days after the last control animal had died.

The peripheral blood of the experimental animals showed more evidence of leukemia than was seen in the experimental animals on the purified diet of Part I. Even with PGA-antagonist at a level of 4% many blast forms appeared although only 2 total leucocyte counts were above 24,000, the maximal normal.

In contrast to the animals on the deficiency regimen of Part I, the histologic findings in the experimental animals of Part II were indistinguishable from those of the controls. Infiltration of all organs was extensive. Very probably this infiltration occurred later and more slowly in the experimental animals as compared to the controls.

There was a moderate loss of weight in some animals; others gained. The mean loss was considerably less than that shown by the animals on the deficiency regimen.

Discussion. Several factors might be considered as having contributed to the experimental results; among these are: 1) simple inanition, 2) deficiency of PGA, 3) an effect of one or more substances in the crude antagonist. Inanition might be seriously considered, if the increased survival times alone were taken into account, and if the paired feeding

technic did not rule out inanition as the determining factor.

If a profound, generalized deficiency of PGA were the determining factor one might expect some evidence for this in the leucocyte picture of the peripheral blood of the experimental animals at the time the controls had florid leukemia. However, no such evidence was found. In this experiment the leucocyte counts were not low at that time and in a preliminary experiment, run under similar conditions, there were no changes in either the white or red blood cell counts. Also, it has been shown in previous experiments[1] that hematologic and histologic evidence of PGA-deficiency do not develop until the animals have been on a purified diet containing succinylsulfathiazole and antagonist for 50 to 60 days. The rate at which PGA-deficiency might develop without the purified diet, as in Part II, has not yet been determined. Of course these findings do not mean that a selective or relative deficiency may not exist. It seems likely that leukemic cells may require a much more abundant supply of PGA than do normal cells.[3] A moderate decrease in available PGA, not enough to produce leucopenia in a normal animal, might be sufficient to decrease the rate of development of leukemic cells. On the other hand, except for the blasts, the leucocytes in the peripheral blood have the appearance of normal cells and merely exist in greater numbers. Perhaps the relative deficiency acts by limiting the excessive production of these essentially normal cells, rather than by interfering with the metabolism of abnormal cells. This reasoning may apply to the cells in the peripheral blood, but does not necessarily explain the marked reduction or complete absence of leukemic infiltration in the liver, spleen, and marrow of the experimental animals in Part I. The infiltrating cells are defintely abnormal as are the blasts of the peripheral blood.

If PGA-deficiency were not the sole explanation for these findings, it is conceivable that an active substance other than the antagonists of PGA may be present in the crude antagonist. However, all growth experiments have indicated that the effects of this material are reversed completely by synthetic PGA.[4,1] Further experiments designed to determine whether PGA-deficiency can account completely for the observed inhibition of the leukemic process are now in progress. These experiments have shown that the leukemic process in mice can be controlled partially by methods and substances now available. Life is prolonged and the severity of the disease is reduced. It is apparent that with the technics so far devised the effects are not of permanent value and the effect on the normal metabolic activity of the animal can be severe. If a profound deficiency of PGA is produced, the leukemia may be controlled, but the treatment eventually is as fatal as the disease. It is logical to expect that this might be true, for it may be assumed that PGA is an essential metabolite utilized in the manufacture of many types of cells and the requirement for the formation of abnormal leucocytes, though larger, probably is not inordinately greater than that for normal cell formation. That the animals of Part I, given a purified diet, developed a deficiency of PGA in the terminal state, is certain. However, additional studies will be required to prove that here also a deficiency of PGA was the only factor operating in the early stage of the experiment when leukemia was suppressed in the experimental groups while the controls had developed florid leukemia. It is our hope that a regimen may be worked out which will provide for selective inhibition of the leukemic process without too seriously impairing the processes essential to the life of normal cells.

Summary. The transplanted leukemia of Ak mice can be partially controlled either by a pteroylglutamic acid-free diet, combined with succinylsulfathiazole and a crude antagonist of pteroylglutamic acid, or by the addition of large amounts of the crude antagonist to an unpurified "normal mouse diet." Life is prolonged and the severity of the disease is reduced.

[3] Bethell, F. H., and Swendseid, M. E., *J. Clin. Invest.*, 1946, **25**, 917.

[4] Franklin, A. L., Stokstad, E. L. R., Belt, M., and Jukes, T. H., *J. Biol. Chem.*, 1947, **169**, 427.

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