had been introduced. In several experiments the aminoguanidine was injected first.

Results. Following injection of formaldehyde, 200 mg/kg aminoguanidine prolonged life slightly but afforded no significant protection. On the other hand 400 mg/kg of aminoguanidine prolonged the life of 60% of the animals more than 24 hours, 20% of the animals being alive after 4 days. When aminoguanidine was administered in doses of 600 mg/kg all animals were alive at the end of a 24-hour period, 60% surviving more than 4 days (Table I). Injection of aminoguanidine previous to the administration of formaldehyde raised these figures slightly. Repeated injection of aminoguanidine was without significant effect.

When methyl alcohol was administered aminoguanidine was without effect in the doses employed. This indicates that the acute toxicity of this substance is due to the depressant effect of the alcohol *per se*, particularly on respiration, and not to the accumulation of formaldehyde. It is possible, however, that some of the effects of chronic methyl alcohol poisoning could be prevented by aminoguanidine.

Summary. Aminoguanidine protects rats against the acute toxic effects of formaldehyde. Following methyl alcohol it is without effect, which indicates that formaldehyde does not contribute significantly to the acute toxicity of this substance.

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Development of Resistance to Folic Acid Antagonists in a Transplantable Lymphoid Leukemia. (17988)

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The development of resistance or insusceptibility to specific substances is a problem of much theoretical and practical interest. The production of resistant microorganisms *in vitro* and *in vivo* to various antimicrobial agents *viz.* antibiotics, antivitamins and bacteriophage has been recorded(1-6). The exact mechanism of this response is not known but has been postulated. It is possible that the development of resistance results from 1) emergence of resistant organisms due to selection, 2) action of the antimicrobial agent on the organism producing a variant, or 3), the development of gene mutations responsible for the resistance but having no direct relationship to the agent used. During the course of studies on the effect of several folic acid antagonists on transplantable acute lymphoid leukemias of the mouse, it has been noted that following a relatively long period of inhibition of leukemic cell growth, there resulted eventually a precipitous exacerbation of the disease with rapid escape of lymphoblasts into the blood, a generalized systemic infiltration into many organs and rapid growth of the localized lymphoma tumor mass in subcutaneously inoculated mice. Thus it was indicated that a resistance to the antifolic compounds had developed despite continued injections of the compounds at levels near the MTD. It was the purpose of this study to 1) attempt to induce resistance in mammalian leukemic cells to PGA antagonists, and 2) to study the characteristics of

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2. Demerec, M., *Jour. Bacteriology*, 1948, v56, 63.

3. Luria, S. E., and Delbrück, M., *Genetics*, 1943, v28, 491.

4. Alexander, H. E. and Leidy, G., *Science*, 1946, v104, 101.

5. Woolley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1944, v55, 179.

6. Youmans, G. P., Williston, E. H., Feldman, W. H., and Hinshow, C., *Proc. Staff Meet. Mayo Clinic*, 1946, v21, 126.

this resistance in the hope of establishing a rational approach to the chemotherapy of leukemia.

The experimental study reported herein relates to the response of 3 separate sublines of acute lymphoid leukemia, L1210(7) in consecutive transplant generations, to the MTD dosage of 4-amino-9-methyl PGA (A-ninopterin), 4 amino-9, N^{10}-dimethyl PGA (A-denopterin) and 4-amino-N^{10}-methyl PGA (A-methopterin) respectively.* Inoculations subcutaneously of leukemic cells into strain dba, subline 212, mice were done by use of a Lockes' solution suspension of spleen, lymph nodes and tumor mass as described previously(8). Beginning with the 60th transplant generation of leukemia L1210 (A-ninopterin and A-denopterin groups) and with the 65th transplant generation (A-methopterin group), the inoculated mice of each experimental series were divided into 2 groups, those treated with the folic acid antagonist and those continued untreated as controls. The estimated MTD for each antagonist, 3 mg/kg, was injected intraperitoneally (.01 cc/g body weight) every other day beginning at 48 hours after inoculation. Injections of the PGA antagonists were continued every other day until the subcutaneous growth of leukemic cells was large enough to transplant. Thus, in the early transfer generations the total dosage of antagonist given was in the order of 24 mg/kg. When resistance of the transplanted leukemic cells to the PGA antagonist developed, the total dosage given in each subline was 12 mg/kg body weight. Transfer of each subline, designated hereafter as AM-resistant, AD-resistant and AN-resistant, was continued through mice receiving the PGA antagonists. An attempt was made in all 3 sublines to transplant the leukemic cells as soon as possible when the growth was large enough to transplant as a brei suspension to at least 10 mice.

Burchenal *et al.*(9) reported the development of resistance of leukemia Ak-4 to repeated doses of 4-amino-N^{10}-methyl PGA using average survival time as the criterion. We have preferred to use a more exact measure of the growth of leukemic tissue and have used throughout the study reported here the mean weight in milligrams at 9 days of the localized lymphoma tissue, following subcutaneous inoculation.

In subline AN-resistant, given 4-amino-9-methyl PGA, no growth of the localized lymphoma mass at 9 days was observed during the first 4 transplant generations. The first palpable growth was found at 18 days in the first 3 transplant generations and at 10 days in the 4th transplant generation. At 9 days the mean weight of the tumor of the 5th transplant generation was 202.9 mg. This subline has now been carried through 13 transplant generations and continues to be resistant to the usual MTD of 3 mg/kg; the localized lymphoma cells growing profusely and attaining a size approximating that of the control line.

Subline AD-resistant treated with the MTD of 4 amino-9, N^{10}-dimethyl PGA remained sensitive to the antagonist through the first 4 transplant generations. At 9 days no growth of the tumor mass was observed; the first palpable growth was detected at 15 days in the first 2 transplant generations, and at 12 days in transplant generations 3 and 4. The mean weight of the lymphoma mass in the 5th generation was 104.9 mg. During the subsequent 10 transplant generations this subline has remained resistant to the usual MTD of 3 mg/kg of 4-amino-9, N^{10}-dimethyl PGA, attaining a mean weight of lymphoma tissue at 9 days of approximately 450 mg.

At the 9th transplant generation in treated mice an additional subline of the AD-resistant group was established by transplantation into dba mice which did not receive this PGA antagonist. This subline has now been carried through 7 generations of untreated mice and remains resistant to therapy with 3 mg/kg of 4-amino-9, N^{10}-dimethyl PGA.

A third subline, subline AM-resistant,

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* Acknowledgement is made to the Lederle Laboratories Division of the American Cyanamid Co. from whom these compounds were obtained.

8. Law, L. W., *Cancer Research*, 1950, v10, 186.

9. Burchenal, J. H., Robinson, E., Johnston, S. F. and Kushida, M. K., *Science*, 1950, v111, 116.

transplanted through dba mice treated with the MTD, 3 mg/kg, of 4 amino-N^{10}-methyl PGA remained susceptible to the antagonist through 5 transplant generations. No growth was present during these passages at 9 days and the first palpable growths were found at 18, 18, 12, 14 and 22 days respectively. Complete resistance was evident in the 6th transplant generation. The mean weight of the lymphoma mass at 9 days was 741.3 mg. This subline has remained resistant, to the present time, through 12 transplant generations, with continued administration of 3 mg/kg of 4-amino-N^{10}-methyl PGA, attaining at 9 days growth comparable to the untreated control line.

Upon development of resistance in all 3 sublines investigated, a pronounced systemic infiltration into subcutaneous and internal lymph nodes, spleen, liver, thymus, kidneys and ovaries and escape of lymphoblasts into the blood stream was evident at 9 days. This is in contrast to the control line which showed no localized growth of lymphoma cells, slight if any systemic infiltration and very few lymphoblasts in the blood at 9 days following a total of 4 injections, every other day of the MTD of any of the 3 PGA antagonists investigated.

There were observed no histologic differences between the resistant and control susceptible tissues examined.

In contrast to the resistance to the PGA antagonists observed in these 3 transplant sublines, identical generations of control L1210 leukemic tissue carried in transplant in untreated strain dba mice have remained susceptible to the MTD of each of these PGA antagonists.

It was found that resistance to each of the antivitamins in the 3 transplant sublines of leukemia L1210 investigated was not independent of resistance to other closely related antagonists. For example, growth of leukemic cells of subline AD-resistant was not inhibited by injections every other day for 4 days of 3 mg/kg of 4-amino-9-methyl PGA nor of 4-amino-N^{10}-methyl PGA. Growth of leukemic cells of subline AM-resistant was not inhibited by the usual therapeutic dose, 20 mg/kg of 4-amino-pteroylaspartic acid, and growth of leukemic cells of subline AN-resistant following treatment with the MTD of 4-amino-9-N^{10}-dimethyl PGA (3 mg/kg) was comparable to the growth obtained following administration of 3 mg/kg of 4 amino-9-methyl PGA. Complete inhibition of the growth of leukemic cells at 9 days was obtained in the comparable transplant generations of the control leukemia in mice given the MTD of each of these antagonists. Determinations of the effects of other specific antileukemic agents on these resistant sublines are now under way.

Further studies with subline AM-resistant leukemia L1210 revealed that leukemic cells of this subline grew better in the presence of the antagonist, 4-amino-N^{10}-methyl PGA than in the absence of the compound. Pteroylglutamic acid had no apparent effect on the growth of leukemic cells of this subline. The growth obtained at 9 days, in this resistant subline, following intraperitoneal injections of 30 mg/kg body weight every other day for 4 days was essentially that of control (untreated) mice and approximately one-half the mean weight of growth in mice given injections of the PGA antagonist (Table I). Considerable variation in mean weights of the lymphoma tumor mass occurred among early transplant generations following the development of resistance indicating that a complete selection of resistant cells had not been attained. In the later transplant generations used variability in growth among the transplant generations was reduced.

A solution of the mechanism responsible for the development of resistance of mammalian cells reported here is probably not possible with the present material. A leukemic cell population originating from a single transplanted cell(10), however, would provide for a study of the mechanism involved. Such studies are now in progress.

Summary. A development of resistance has been shown in 3 separate sublines of a transplantable lymphoid leukemia, L1210, in strain dba mice following successive transplants in mice treated with the MTD of 3 different PGA antagonists: 4-amino-N^{10} methyl PGA,

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TABLE I.
Effect of 4-amino-N10 Methyl-pteroylglutamic Acid and Pteroylglutamic Acid on Subline AM-resistant Lymphoid Leukemia L1210.

Experiment	Daily dosage, mg/kg	Transplant generation	No. of mice	Mean wt of tumor, mg*	Absolute No. lymphoblasts (peripheral blood)
AM-Resistant L1210					
4-amino-N10-methyl-pteroylglutamic acid	3.0		38	388.5	1430†
Pteroylglutamic acid	30	G7 to G11	34	159.0 } 176.7	—
Controls	—		20	207.7 }	—
Diff. of means = 211.8 ± 54.6 mg; t = 3.9; P >0.01‡					
4 amino-N10-methyl-pteroylglutamic acid	2.5	G12	12	750.2	
Controls	—	"	13	336.5	
Diff. of means = 413.7 ± 59.2 mg; t = 6.9; P >0.001					
Control L1210					
4 amino-N10-methyl-pteroylglutamic acid	3.0	G1 to G6	25	No growth	
Pteroylglutamic acid	30	G6	5	544.0	
Controls	—		5	494.3	
4 amino-N10-methyl-pteroylglutamic acid	2.5		5	28.6	300
Pteroylglutamic acid	30	G12	5	581.7	—
Controls	—		5	808.1	1750

* Wet wt obtained on a Roller-Smith micro torsion balance.
† The mean absolute number obtained from 6 mice, transplant generations 6, 7, and 8.
‡ Since the difference of the mean weights of the tumor mass in the pteroylglutamic acid group and the control group was not significant statistically, the mean weight of pooled data for these 2 groups (176.7) mg is compared with the mean weight of the 4-amino-N10-methyl-pteroylglutamic acid group.

4-amino-9, N^{10} dimethyl PGA and 4-amino-9 methyl PGA. All 3 sublines continue to be resistant in subsequent transplant generations following emergence of resistance. Subline AD-resistant leukemic cells grown in mice not given the folic acid antagonist for 7 successive transplant generations continue to be resistant to 4-amino-9, N^{10} dimethyl PGA. Resistance developed to one of these antivitamins was found not to be independent of resistance to the others. AM-resistant leukemic cells were found to grow better in animals receiving the MTD of 4-amino-N^{10} methyl PGA than in animals untreated with this antivitamin or given PGA parenterally.

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Diabetic State with Lipaemia and Hydropic Changes in the Pancreas Produced in Rabbits by Cortisone.* (17989)

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During the course of an experiment undertaken with the purpose of observing the effect of Cortisone on the tissue lesions produced by foreign serum proteins in rabbits living in a cold environment, a marked alteration of carbohydrate metabolism which resembled a diabetic state and abnormalities of the Islets of

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