changed and the birefringence with square waves should be equal to the steady state value with an equivalent steady field, and this is consistent with our observations. Because the conductivity of the dispersing medium will affect the ionic atmosphere and the electric field in the vicinity of a particle, a change of birefringence with buffer concentration is to be expected. The observed result, a decrease in birefringence with increasing buffer concentration, is illustrated in Fig. 2, curves a and b. The complex nature and reversal of sign of the birefringence at higher concentrations (curve c) might be due to interactions between the particles and their ionic atmospheres.

Whatever may be the orienting mechanisms involved, it is clear that the birefringence and its decay are very sensitive to the size and shape of the particles. For this reason, the method shows promise of application in aggregation studies and other investigations in which the size of large colloidal particles is important.

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The Induction of Resistance to 4-Amino-N^{10}-Methyl-Pteroylglutamic Acid in a Strain of Transmitted Mouse Leukemia[1]

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It has been shown previously (*2, 3*) that there is a marked prolongation of the survival time of mice inoculated with transplanted leukemia Ak 4 when they are treated with 3 mg/kg of 4-amino-N^{10}-methyl-pteroylglutamic acid[2] (*6*) three times weekly to a total of ten doses. Even when treatment is continued until death, however, the mice eventually die of leukemia between the 28th and 40th day. In view of the ultimate failure of therapy observed with this and closely related substances in many clinical trials in acute leukemia, the mechanism of this eventual lack of response in mouse leukemia was deemed worthy of further investigation.

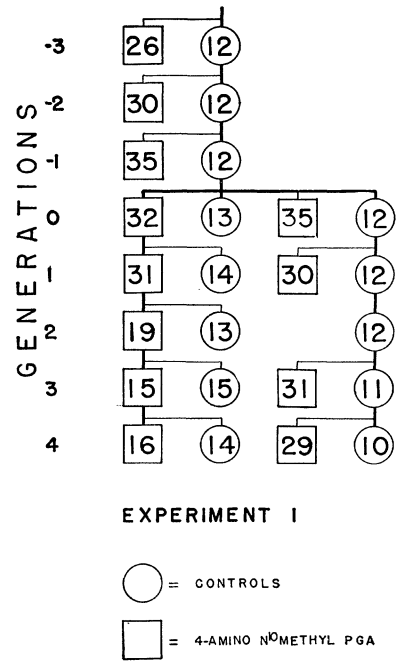

FIG. 1. Genealogy of 4-amino-N^{10}-methyl PGA-resistant strains. The figures in the circles represent the average survival time in days of groups of ten untreated mice; those in the squares the average survival time of groups of ten mice treated with 4-amino-N^{10}-methyl-PGA 3 mg/kg intraperitoneally three times weekly.

Many examples of the development of drug-fast strains in microorganisms under drug treatment *in vitro* and *in vivo* have been noted in the history of chemotherapy (*1, 4, 7*). Unpublished work elsewhere has shown a similar drug fastness to develop in cells of tumors of chloroleukemia Ak 1394 in mice treated with benzene (*5*).

The experimental studies undertaken to develop such a drug-resistant subline in mice are herewith reported. Akm mice inoculated with leukemia Ak 4 in from the 21st to the 35th transplanted generation were used in these experiments. Saline suspensions of splenic tissue, which had been obtained from mice dying of leukemia Ak 4 despite continued therapy with 4-amino-N^{10}-methyl-PGA in doses of 3 mg/kg given three times weekly, were inoculated intraperitoneally into 20 Akm mice. Forty-eight hr later these were divided into two groups of ten mice each. One group was considered as a control and received no treatment. The other group was treated with 4-amino-N^{10}-methyl-PGA in doses of 3 mg/kg given intraperitoneally three times weekly until death. The average survival time of treated and control mice was noted and transfer of the line continued through one of the treated mice. The genealogy and differing response to therapy of sublines after repeated passages through treated or untreated mice can be seen in Fig. 1.

In experiment 1, a subline of this lukemia developed complete resistance to 4-amino-N^{10}-methyl-PGA after three

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[2] We are indebted to Dr. J. H. Williams of the Lederle Laboratories for our supply of this compound.

passages through treated mice. This can be contrasted with identical generations in which passage of the leukemia was continued through the untreated mice. In these, as would have been expected, the sensitivity of the disease to therapy continued unchanged (Fig. 1). In a second experiment using the same original strain of Ak 4 leukemia, a marked drop in the survival time of the treated mice occurred after the first passage through treated mice. For the next two passages no further increase in resistance was noted, but after the fourth transfer a drug-fast strain developed which showed no significant difference in the survival time of treated and untreated mice. Both groups died approximately 12 days after the inoculation of the leukemia. This subline is now in the ninth transfer generation through treated mice. All continue to be resistant to the usual therapy with 4-amino-N^{10}-methyl-PGA. This procedure has been repeated with a third subline with similar results.

No morphologic differences between the cells of the sensitive and of the resistant sublines of this leukemia have been observed, and sections taken at the time of death from mice inoculated with the normal or the resistant sublines were indistinguishable. Studies are in progress in an attempt to demonstrate biochemical differences between these cells.

It is felt that this demonstration of the ability of a hitherto sensitive leukemic strain to develop resistance to 4-amino-N^{10}-methyl-pteroylglutamic acid may help to explain the eventual failure of this type of therapy in patients with acute leukemia.

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Hemin Synthesis in Spleen Homogenates

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It has been demonstrated by Altman *et al.* (*2*) that the alpha carbon atom of glycine is incorporated in the hemin and globin moieties of the hemoglobin molecule when glycine labeled with C^{14} in the methylene carbon atom is fed to rats. Several instances of hemin synthesis *in vitro* are known. It has thus been shown that hemin synthesis from methylene carbon-labeled glycine takes place in rabbit bone marrow homogenates (*3*). It has also been shown that nucleated avian erythrocytes are capable of hemin synthesis *in vitro* when glycine labeled with N^{15} is added to the incubation mixture (*9*). Since there exists histological evidence of extra-medullary hematopoietic activity in the spleen (*4, 7*), it was thought of interest to test with biochemical methods the possibility of hemin synthesis from labeled glycine in spleen homogenates in the manner previously applied to bone marrow homogenates (*3*).

Rabbit spleens were chosen as the source of the homogenates, spleens from several rabbits having been pooled for each experiment. A spleen homogenate from three rabbit spleens, 8–10 g wet weight in toto, was prepared as follows: The spleens were homogenized in the microcup of the Waring blender with 25 ml of 0.9% NaCl solution. To the resulting homogenate were added 0.15 millimoles of glycine (containing concentrations of $C^{14}H_2NH_2COOH$[2] indicated in Table 1), 0.06 millimoles of sodium acetate, and 1.5 ml of M/2 phosphate buffer pH 7.3. The homogenates were then incubated at 38° C for appropriate periods of time. After addition of 10 mg crystalline hemin as carrier, either hemin or protoporphyrin IX dimethyl ester was isolated, hemin according to Nencki and Zaleski (*8*) and protoporphyrin according to Grinstein (*6*). The protoporphyrin dimethyl ester was recrystallized three times from chloroform and once from pyridine (mp 223–225°). Hemin was recrystallized once as described by Fischer (*5*). The determination of C^{14} activity was carried out with the ionization chamber apparatus of Bale and Masters, as previously described (*2*). The results obtained are shown in Table 1.

TABLE 1

Time of incubation in hr	C^{14} activity of glycine 10^6 disintegrations/min/millimole	C^{14} activity of protoporphyrin IX dimethyl ester 10^4 disintegrations/min/millimole	C_o/C†
3	4.6	16.4	28.0
14½	7.2	48.8	14.8
25	5.3	60.0*	8.8

* Isolated as hemin.

† C_o/C = ratio of C^{14} activity of compound added (C_o) to C^{14} activity of compound isolated (C), i.e., the dilution constant.

One experiment (3-hr incubation) was carried out in a large Warburg vessel permitting the collection of evolved CO_2 in 5*N* NaOH with subsequent isolation as $BaCO_3$. The C^{14} activity of the $BaCO_3$ thus obtained was quite low (4.8×10^2 disintegrations/min/millimole), indicating that only very small amounts of the methylene carbon atom of glycine were converted to CO_2 in spleen homogenates. The C_{16}–C_{18} fatty acids isolated in several cases contained significant C^{14} activities, although the dilution of the radioactivity was somewhat higher than that pre-

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