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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viously reported for bone marrow homogenates (*1*). Active protein and nucleic acid synthesis also appeared to take place in these spleen homogenates.

The results reported here indicate that hemin synthesis can be carried out by rabbit spleen homogenates utilizing the methylene carbon atom of glycine as a precursor. Experiments are now in progress to assess the biological significance of these findings.

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Effect of 2,4-Dichlorophenoxyacetic Acid on the Alpha and Beta Amylase Activity in the Stems and Leaves of Red Kidney Bean Plants[1]

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Recent work (*2, 4, 5, 7, 8*) has shown that treatment of plants with 2,4-dichlorophenoxyacetic acid (2,4-D) results in a reduction of carbohydrates and an accumulation of nitrogen. Since minute quantities of 2,4-D produce these marked changes in the chemical composition of the plants, it is indicated that the enzyme system might be involved. The purpose of this communication is to report the effect of 2,4-D on the alpha and beta amylase activity in the stem and leaf tissue of red kidney bean plants.

Seeds of red kidney bean plants were selected for uniformity of size and planted in 4-in. pots in the greenhouse. Each pot contained two plants that were treated when the first trifoliate leaf was expanding. Four replications of 100 plants each were used from which to obtain material of treated and nontreated plants (controls). Application of 2,4-D was made by applying one drop (0.05 ml) of a 1000-ppm solution to the base of the blade of one of the primary leaves. The plants were harvested 6 days after treatment, at the time the stem tissue had proliferated considerably but yet showed no signs of necrosis. The plants were air-dried in the dark and then separated into the various parts. The hypocotyl, first internode, and leaf petioles were grouped together as stem tissue. Enzyme activity was determined separately on the stem and leaf tissue.

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TABLE 1

EFFECT OF 2,4-D ON ALPHA AND BETA AMYLASE ACTIVITY IN STEMS AND LEAVES OF RED KIDNEY BEAN PLANTS*

Enzyme	Replication	Stems		Leaves	
		non-treated	treated	non-treated	treated
Alpha amylase†	1	29.91	4.63	0	0
	2	31.90	4.90	0	0
	3	32.32	5.02	0	0
	4	33.20	5.23	0	0
	avg	31.83	4.95	0	0
Beta amylase‡	1	38.70	21.15	25.87	27.91
	2	33.40	25.99	26.20	26.90
	3	35.60	26.22	27.62	28.34
	4	38.60	22.34	25.62	26.34
	avg	36.58	23.93	26.33	27.37

* Each figure is the average of two determinations of each replication. Results are expressed on a dry weight basis.

† Expressed as the number of grams of soluble starch which under the influence of an excess of beta amylase are dextrinized by 1 g of tissue in 1 hr at 30° C.

‡ Expressed as number of grams of soluble starch converted to maltose by the beta amylase of 1 g of tissue in 1 hr at 30° C.

The alpha and beta amylase were determined according to the method of Kneen and Sandstedt (*1, 3, 6*). The data in Table 1 show that 2,4-D lowers considerably the activity of both the alpha and beta amylase in the stems of bean plants. No activity of alpha amylase was noted in leaves of the treated and nontreated plants. The results also show that treating leaves of the plants with 2,4-D had no effect on the beta amylase activity.

Further work is in progress on the effect of 2,4-D upon enzyme activity in various tissues of the plant.

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