Glycine Inhibition of Asparaginase

Abstract. *Administration of either* Escherichia coli *asparaginase or guinea pig serum to C3H/HE mice with the 6C3HED lymphosarcoma is followed by depression of glycine in the tumor. This decrease in cellular glycine concentration does not occur in a tumor resistant to asparaginase. The inhibition of the lymphosarcoma by asparaginase can be reversed by intraperitoneal injection of asparagine or glycine. This reversal appears to be specific because lysine, threonine, serine, and aspartic acid were ineffective. Loss of cellular glycine may be more important than loss of asparagine because of the requirement for glycine in purine synthesis.*

After inhibition of growth of lymphomas by guinea pig serum was demonstrated (*1*), Broome showed that the inhibition is due to asparaginase (E.C. 3.5.1.1) in the guinea pig serum (*2*). Subsequently an asparaginase which also inhibits lymphoma growth was isolated from *Escherichia coli* (*3*). It is not certain how asparaginase inhibits lymphosarcomas. The explanation that asparagine depletion inhibits tumor growth is inadequate because the cellular concentrations of asparagine are equally depressed in normal tissues and resistant tumors. There is therefore no correlation between asparagine concentration and cellular damage (see *4*).

Measurement of all of the amino acids of normal tissues, of susceptible tumors, and of resistant tumors after injection of *E. coli* asparaginase indicated that there was only one amino acid change which was unique to the susceptible tumor. Glycine decreases in the susceptible tumor but not in the resistant tumor or the normal tissues (*5*).

Because of the difference in activity of guinea pig serum asparaginase and *E. coli* asparaginase (*6*), we compared the effects of both enzymes on glycine concentrations in the lymphosarcoma and measured the ability of glycine and asparagine to reverse the inhibiting effects of *E. coli* asparaginase on the 6C3HED lymphosarcoma.

Mice (C3H/HeJ) were inoculated subcutaneously with 100,000 lymphosarcoma cells (6C3HED or 6C3HED resistant) (*1*). Seven days after tumor inoculation, asparaginase from *E. coli* and guinea pig serum was administered intraperitoneally. The tumors were removed at 0, 24, and 48 hours. They were weighed, frozen with solid CO_2, and prepared for amino acid analysis and analyzed on a Spinco 116 amino acid analyzer (*7*) (Table 1). Glycine decreased significantly in the tumors susceptible to asparaginase whether treated with asparaginase from *E. coli* [15 international units (I.U.) per animal] or from guinea pig serum (1 I.U. per animal). However, the glycine concentration in the resistant tumor rose significantly after treatment with *E. coli* asparaginase.

Several investigators have tried unsuccessfully to reverse the tumor regression caused by asparaginase by injecting asparagine (*8*, *9*). Asparagine administered in the drinking water increased the growth of tumors in mice given a low concentration of asparaginase (*10*). Under appropriate conditions, it is possible to completely inhibit the effects of asparaginase by injection of asparagine (Fig. 1). Glycine at the same molar concentration and dosage schedule also antagonized the effects of asparaginase in 50 percent of the 20 mice. The weight curve in the mice receiving glycine rose and fell because 4 of the 20 mice had tumors that regressed only slightly. These mice died on days 17 and 18. An additional six mice lost nearly all of their tumor when treated with asparaginase and glycine, and the tumor grew back very slowly. The latter group died from their tumors on days 26 and 27. Both glycine and asparagine antagonized the effects of asparaginase. Only partial inhibition of the effect of asparaginase on the 6C3HED lymphosarcoma by glycine was achieved; however this may be because the optimum dosage schedule for glycine differs from that of asparagine.

Experiments with aspartic acid, threonine, and lysine under the same conditions as described in Fig. 1 resulted in no regrowth of the tumors treated with asparaginase. In two experiments with serine (20 mice in each experiment) under the same conditions, only one mouse had a tumor which did not regress after treatment with asparaginase.

In previous studies asparaginase treatment of the 6C3HED lymphosarcoma decreased only the amino acids glycine and asparagine (*5*). Our experiments show that the decrease in glycine is limited to the susceptible tumor and is produced by asparaginase from either *E. coli* or guinea pig serum. Also, the administration of glycine during asparaginase treatment antagonized the inhibitory effects of the enzyme on the tumor. These data suggest that glycine plays an important and perhaps unique role in asparaginase inhibition of lymphomas. The decrease in glycine may arise because the tumor synthesizes cellular glycine from glyoxylic acid and asparagine. Synthesis of glycine in the liver by this route has been shown by Meister (*11*). The deprivation of asparagine by way of asparaginase would therefore deprive the tumor of glycine.

Table 1. Glycine concentration in the 6C3-HED tumor after treatment with asparaginase from guinea pig serum and *E. coli*. The mice were injected with the indicated amounts of asparaginase 7 days after tumor transplantation. The results are expressed as micromoles of glycine per gram (wet weight). The values are an average of five determinations.

Time (hr)	Susceptible tumor		Resistant tumor
	Guinea pig serum (1 I.U.)	*E. coli* (15 I.U.)	*E. coli* (15 I.U.)
0	8.1	7.6	6.8
24	5.6*	4.1*	8.4
48	7.6	2.3*	9.1*

* Significant by Mann-Whitney U test when compared to untreated group ($P < .05$).

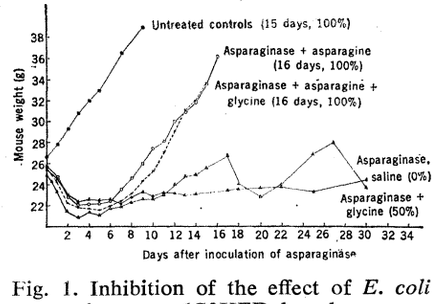

Fig. 1. Inhibition of the effect of *E. coli* asparaginase on 6C3HED lymphosarcoma by asparagine and glycine. There were 20 mice in each group; into each 15 I.U. of *E. coli* asparaginase was injected subcutaneously 7 days after tumor inoculation. Seven days after tumor inoculation the amino acids were injected intraperitoneally four times a day at intervals of 4 hours. A total of 0.8 mmole, divided equally, was injected per day for 5 days. The numbers in parentheses show the average day of death and percent of mice dead from the tumor.

The usual conversion of serine to glycine may be available to the normal tissues and the resistant tumor, and for this reason asparaginase affects these tissues very little. The importance of glycine in purine synthesis may explain why the lack of glycine which occurs only in the susceptible tumor is more damaging to cells than the lack of asparagine which occurs in all tissues after asparaginase treatment.

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Heterozygous Beta Thalassemia: Balanced Globin Synthesis in Bone Marrow Cells

Abstract. *In two patients with heterozygous beta thalassemia the rates of synthesis of the alpha and beta chains of hemoglobin were equal in nucleated red cell precursors, although beta chain synthesis was reduced in peripheral blood reticulocytes. This finding suggests a relative instability of beta chain messenger RNA in beta thalassemia.*

A marked imbalance of globin chain synthesis occurs in the immature red cells of the peripheral blood of patients with homozygous beta thalassemia (Cooley's anemia) (*1*). Production of normal beta chains is decreased or absent, resulting in a relative excess of normal alpha chains. Reduced synthesis of beta chains also occurs in the peripheral blood of patients heterozygous for beta thalassemia (A_2 thalassemia) (*1*). Defective control of globin synthesis in thalassemia can be shown in experiments involving incubation of peripheral blood red cells with radioactive amino acids and measurement of radioactivity incorporated into the separated globin chains. In nonthalassemic controls with reticulocytosis, equal amounts of radioactivity are incorporated into alpha and beta chains, and the specific activities (radioactivity per absorbance unit) of the two chains are also equal. In patients heterozygous for beta thalassemia, both radioactivity and specific activity of beta chains are approximately one-half those of alpha chains. The difference in uptake of radioactivity between the globin chains indicates that beta chain synthesis proceeds at a slower rate than alpha chain synthesis in peripheral blood reticulocytes of these patients. Studies by Bank have failed to reveal evidence of a significant excess of alpha chains in hemolyzates from patients with heterozygous beta thalassemia (*2*). The relative decrease in synthesis of beta chains in reticulocytes is thus not accompanied by a corresponding disproportion in total amounts of beta and alpha chains in the peripheral blood red cells, indicating a difference between relative net synthesis of alpha and beta chains in nucleated red cells and reticulocytes in heterozygous beta thalassemia. My study was designed to compare the synthesis of globin chains of bone marrow with that of peripheral blood in this disorder.

The relative amounts of globin chains in the peripheral blood reflect mainly synthesis of globin by nucleated red cells in the bone marrow and any premature unequal removal of globin chains. The rates of synthesis of alpha and beta chains may be approximately equal in the bone marrow of patients with heterozygous beta thalassemia during the major period of globin synthesis, with the observed decrease in beta chain synthesis appearing late in red cell maturation. Alternatively, if the decreased rate of synthesis of beta chains in the peripheral blood were also present to the same degree in the nucleated red cell precursors, an intramedullary loss of approximately one-half of the alpha chains produced would be necessary for equal amounts of the chains to be present in the peripheral blood. In the homozygous condition (Cooley's anemia), loss of alpha chain occurs both in vivo and in incubated cells by precipitation and attachment to the membrane (*3*). The cells with inclusions are preferentially destroyed, presumably because of membrane damage (*4*). Those cells that are able to make significant amounts of gamma chain are better able to survive, since alpha chain precipitation is prevented by the formation of hemoglobin F($\alpha_2\gamma_2$). A comparison of globin synthesis in nucleated precursors and reticulocytes in Cooley's anemia has been attempted (*5*) but is difficult to interpret because of the selective destruction of cells with the most significant thalassemia defect in this disorder. A similar comparison in heterozygous thalassemia would be more critical, particularly in patients without intracellular inclusions in the marrow and with normal amounts of hemoglobin F. The following experiments compare globin synthesis in the peripheral blood and marrow of two such patients. Both patients had mild anemia and reticulocytosis, hypochromia, microcytosis, and elevated levels of Hb A_2.

Peripheral blood samples were incubated with L-[^{14}C]leucine (uniformly labeled) for 2 hours. The red cells were washed and lysed, and the alpha and beta chains were separated by chromatography on carboxymethyl cellulose in 8*M* urea (*6*). The absorbance of each fraction was determined at 280 nm, and radioactivity was measured in a liquid scintillation spectrometer.

Table 1. Synthesis of alpha and beta chains in reticulocytes and nucleated red cells from patients heterozygous for beta thalassemia.

Subject	Ratio of beta to alpha	
	Specific activity (count/min per O.D.)	Total radioactivity (count/min)
	Peripheral blood	
C.D.	0.55	0.57
G.H. No. 1	0.62	0.55
	Bone marrow	
C.D.	0.89	0.94
G.H. No. 1	0.94	1.02
G.H. No. 2	0.94	0.98