Serine Requirement in Leukemic and Normal Blood Cells

Abstract. *In a serine-deficient medium, cells from human chronic granulocytic leukemia and normal human bone marrow exhibited a marked inhibition of incorporation of radioactive precursors of DNA, RNA, and protein into an acid insoluble cell fraction. Normal diploid human fibroblasts did not exhibit inhibition without serine. Chronic granulocytic leukemia and normal marrow cells were essentially unable to synthesize C^{14}-serine from C^{14}-glucose, while human diploid fibroblasts were highly capable of this synthesis.*

Some leukemic cells of animals and humans have a requirement for one of the nonessential (*1*) amino acids, namely asparagine [for a review, see (*2*)].

In this report we present evidence which indicates that leukocytes from patients with chronic granulocytic leukemia (CGL) and normal human bone marrow cells exhibit not only a partial asparagine requirement but also a serine requirement that is apparently more severe than the asparagine requirement.

For experiments with CGL cells, peripheral blood was allowed to settle at room temperature for 1 to 2 hours. Most patients had decidedly elevated leukocyte counts; thus sufficient leukocytes could be removed without disturbing the erythrocytic layer. Differential counts showed that 90 to 95 percent of the cells were of the granulocytic series. The cells were centrifuged and the plasma was decanted. The cells were washed once in medium 9 (see Table 1), centrifuged again, resuspended in medium 9, counted, and placed in 50-mm plastic petri dishes, in the appropriate medium, at a concentration of 4 to 5 $\times$ 10^5 cells per milliliter.

Normal bone marrow was collected from donors by iliac puncture. While many mature erythrocytes were present in such a sample, all calculations were based on the number of nucleated cells in the sample. A typical differential count of marrow cells was as follows: myeloid cells, 60 percent; erythroblasts, 29.5 percent; lymphocytes, 6.0 percent; eosinophils, 4.0 percent; and basophils, 0.5 percent.

The cells were incubated in Eagle's minimal essential medium [Hanks base (*1*)] with 10 percent dialyzed (against 30 volumes of normal saline, with three changes at 4°C for 48 hours) calf serum (E_{90}HDCS) at 37°C in a humidified atmosphere containing 2 percent CO_2. The E_{90}HDCS was supplemented with nonessential (*1*) amino acid stock solutions so that each amino acid was present at a final concentration of 1 $\times$ $10^{-4}M$.

DNA and RNA synthesis was measured by incorporation of tritiated thymidine (specific activity, 1.9 c/mmole at 1 μc/ml) and tritiated uridine (specific activity, 2 c/mmole at 1 μc/ml) into a fraction insoluble in trichloroacetic acid, by use of a modification (*3*) of the filter-disc method of Bollum (*4*). Protein synthesis was measured by a similar technique (*5*) with C^{14}-valine or C^{14}-leucine (specific activity, 100 to 200 c/mmole at 0.1 to 0.2 μc/ml). The radioactive precursors were present from 0 to 24 or 0 to 48 hours. The number of cells was determined by an electronic counter.

The synthesis of C^{14}-serine from C^{14}-glucose in chronic granulocytic leukemia cells, in bone marrow cells,

Table 1. Mean percentages for incorporation of radioactive precursors into DNA, RNA, and protein in human chronic granulocytic leukemia cells in media supplemented with amino acids. Medium 1 (complete medium) contained all the nonessential amino acids and was set equal to 100 percent. The variances in each column were homogeneous and hence could be pooled; thus one SEM applies to all percentages of that column. Ninety-five percent confidence limits = $\pm$ 2 $\times$ SEM (SEM, standard error of the mean).

Medium	Nonessential amino acid omitted	^{3}H-Thymidine (%)		^{3}H-Uridine (%)		^{14}C-Amino acid (%)	
		24 hours (SEM 6.4)	48 hours (SEM 7.5)	24 hours (SEM 6.6)	48 hours (SEM 5.8)	24 hours (SEM 5.3)	48 hours (SEM 4.7)
2	Alanine	90.5	100.3	93.7	101.6	90.8	99.0
3	Aspartic acid	97.1	97.8	102.7	102.9	102.3	92.5
4	Asparagine	53.9	63.8	79.1	84.7	80.5	71.9
5	Glycine	95.6	94.9	101.2	91.5	101.7	94.8
6	Glutamic acid	105.5	102.4	103.9	96.5	104.2	98.3
7	Proline	94.0	104.7	105.2	95.0	95.5	91.6
8	Serine	40.8	40.8	66.4	50.9	67.1	56.9
9	All	40.6	32.3	66.2	54.8	64.6	55.2

Table 2. Mean percentages for incorporation of radioactive precursors into DNA, RNA, and protein in normal human bone marrow cells in media supplemented with amino acids. For statistical treatment see Table 1.

Medium	Nonessential amino acid omitted	^{3}H-Thymidine (%)		^{3}H-Uridine (%)		^{14}C-Amino acid (%)	
		24 hours (SEM 4.8)	48 hours (SEM 4.1)	24 hours (SEM 4.0)	48 hours (SEM 3.0)	24 hours (SEM 4.0)	48 hours (SEM 3.4)
2	Alanine	99.6	99.8	101.4	102.8	94.2	96.9
3	Aspartic acid	97.3	100.6	100.6	101.7	102.5	101.5
4	Asparagine	63.7	77.0	81.1	87.2	72.7	74.5
5	Glycine	101.7	103.0	104.4	101.5	96.9	103.5
6	Glutamic acid	102.2	102.6	107.8	104.0	100.8	100.8
7	Proline	100.4	100.8	102.6	102.5	97.1	101.9
8	Serine	46.5	57.6	66.1	71.1	60.1	61.5
9	All	47.5	57.8	66.1	70.4	61.9	63.6

and in WI-38 human diploid fibroblasts (*6*) was studied by incubating the cells for 48 hours in medium 9 (Table 1), to which was added 0.5 mc of C^{14}-glucose (specific activity, 15 mc/mmole). The cells were then harvested by scraping and centrifugation and subjected to conditions employed for the total hydrolysis of protein (6*N* HCl in evacuated sealed tubes at 110°C for 21 hours). Samples of the concentrated hydrolyzates were analyzed on the long column of an automatic amino acid analyzer (*7*). The serine peak was collected and its radioactivity determined by liquid scintillation counting.

Table 1 shows mean percentages for incorporation of radioactive precursors of DNA, RNA, and protein during 24- and 48-hour labeling periods. These data are derived from nine different experiments with six CGL patients. All but one of the patients had received treatment; this factor was ignored in our studies since it did not seem to affect the amino acid requirements of the cells. Partial inhibition of incorporation occurred because of the lack of asparagine (medium 4); however, the lack of serine (medium 8) was more inhibitory to incorporation and was similar to the effect produced by minimal medium (medium 9). The results with media 2 through 7, except 4, do not differ significantly from 100 percent; results with media 8 and 9 are significantly different from results with the other media. Experiments with cell concentrations of 1×10^5 to 15×10^5 cells per milliliter showed that the serine requirement was not dependent on cell density. We have also examined cells from three patients with acute granulocytic leukemia; the results were essentially the same as with CGL cells.

Table 2 shows the results of similar experiments with four different samples of normal bone marrow cells. In the absence of either asparagine or serine the cells exhibited a degree of metabolic inhibition. Again, the effect due to the lack of serine was more pronounced than that due to the lack of asparagine.

Experiments similar to those reported in Table 2 were performed with WI-38 (*6*) human diploid fibroblast cells. These cells were not inhibited by the lack of any of the nonessential amino acids.

In a glucose-containing culture medium, human cells that are capable of synthesizing serine use glucose almost exclusively to form the carbon skeleton of serine (*8*). We therefore examined the synthesis of C^{14}-serine from C^{14}-glucose in CGL cells, in normal marrow, and in WI-38 cells under similar conditions. Results in terms of radioactivity in total cellular serine for the three cell types were: CGL cells, 2100 count min^{-1} $\mu mole^{-1}$; bone marrow, 1395 count min^{-1} $\mu mole^{-1}$; and WI-38 cells, 317,700 count min^{-1} $\mu mole^{-1}$. Thus, while WI-38 cells could readily synthesize C^{14}-serine from C^{14}-glucose, CGL cells and normal bone marrow cells were only minimally capable of this synthesis.

Both the incorporation data reported in Tables 1 and 2 and the minimal C^{14}-serine synthesis from C^{14}-glucose seem to suggest that, although bone marrow and CGL cells may have a partial asparagine requirement, they also have a serine requirement that appears to be more severe.

Our results suggest that normal human bone marrow cells and cells derived therefrom, whether leukemic or not, may require preformed serine. If true, it is conceivable that therapeutic measures involving the enzyme serine dehydratase or serine antimetabolites could be beneficial in leukemia, providing the in vivo growth rate of the leukemic cells is sufficiently elevated above that of normal bone marrow cells.

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Lymphocytoid Lines from Persons with Sex Chromosome Anomalies

Abstract. *Permanent lymphocytoid cell lines have been established from patients with the XYY and XXY chromosome constitutions. Large amounts of these and other cell lines with genetic defects can be provided for various studies.*

A series of technical innovations have made possible the discovery and study of many genetic abnormalities in temporary cultures of human cells. For example, Jacobs and Strong (*1*) described cells with an extra chromosome in Klinefelter's syndrome, and Hauschka *et al.* (*2*) reported the XYY anomaly. Subsequently a large number of anomalies of the sex chromosomes have been described (*3*).

Seemingly permanent human lymphocytoid cell lines have been established from patients with various malignant and nonmalignant diseases (*4*) and from normal persons (*5*). Over 500 such cell lines have been initiated in our laboratory. The cell lines have been valuable for viral, biochemical, immunological, and clinical studies. An effort is now being made to establish a library of lymphocytoid cell lines from patients with chromosome anomalies and other genetic abnormalities.

A mentally retarded 28-year-old man with antisocial behavior and a history of suspected arson had an XYY phenotype (*6*). Leukapheresis of 500 ml of peripheral blood was performed on 24 July 1968, and six cultures were initiated by techniques reported previously (*7*). On 16 September 1968 two cultures began to grow rapidly and were considered established; they were labeled RPMI cell lines No. 1608 and No.1618. These cell lines had a 46 XYY chromosome constitution (plus an extra Y) and have continued to grow vigorously.

Leukapheresis was performed on blood from a 31-year-old man with Klinefelter's syndrome. Eight cultures were started on 2 July 1968; after several cultures were combined, one culture began growing rapidly on 21 October