

# Glutamic Acid Dehydrogenase and Glutamic-oxalacetic Transaminase of Blood in Leukemia and Cancer\*

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As part of a larger project dealing with an investigation of amino acid and protein metabolism in leukemia, a study was undertaken to determine those enzymes concerned with amino acid anabolism and catabolism as they occur in selected tissues. Several review papers (2, 4, 11, 12) have adequately summarized our present knowledge of the metabolism of white blood cells (WBC) in leukemia. However, few efforts have been made to study the enzymes related to amino acid metabolism in WBC. As an initial investigation we have isolated WBC from leukemic patients and from normal individuals and have examined their content of glutamic acid dehydrogenase (GAD) and glutamic-oxalacetic transaminase (GOAT), two of the enzymes concerned with glutamic acid metabolism.

## MATERIALS AND METHODS

Blood samples were drawn in syringes moistened with 1:5000 heparin from patients at University Hospitals. Volunteers from the laboratory or hospital staff provided blood for the normal control group. The diagnosis of leukemia was confirmed in all cases by evaluation of the bone marrow by one of us (H. A. W.) or by the hematology laboratory. The diagnosis of cancer was made from biopsy of the suspected lesion or from the surgical specimen. Complete blood counts were made at the time the blood was drawn.

Successful separation of WBC from other blood constituents was obtained by allowing the red blood cells (RBC) to settle by gravity in sterile centrifuge tubes or after inducing rouleaux formation with 0.8 (wt/vol) per cent dextran. Seventy to 80 per cent of the WBC could be recovered in the supernatant plasma in this way. Although the WBC in these preparations were usually con-

taminated with approximately equal numbers of RBC, interference from the RBC was not encountered in these studies, since they contained no detectable GOAT or GAD. The upper layer, containing plasma, platelets, RBC, and WBC, was removed by aspiration. The WBC were separated from the plasma after centrifugation at  $1300 \times g$  for 10–15 minutes and washed twice with physiological saline (NaCl) solution. Saline was added to the packed cells to give a final concentration of  $10^7$  WBC per 0.1 ml. suspension.

Since intact WBC exhibited no GAD or GOAT activity, several technics for disrupting the cells were investigated. Homogenizing the cell suspension in a Potter-Elvehjem glass homogenizer disrupted only about 25 per cent of the cells. Laking the cells in distilled water also gave no enzyme activity. Freezing and thawing, however, completely disrupted the cells, resulting in high GAD and GOAT activity. In the procedure finally adopted, the WBC suspension in saline was frozen, thawed, and homogenized for three cycles, then centrifuged at  $1300 \times g$  for 10 minutes. GAD in the supernatant was determined by measuring the reduction of added DPN at  $340 \text{ m}\mu$  (Beckman Model DU Spectrophotometer) in a system containing 1.1 ml. 0.5 M potassium phosphate buffer (pH 7.6), 0.6 ml. 0.5 M potassium glutamate (pH 7.6), 0.1 ml. of 1 per cent DPN, and the extract from  $2 \times 10^7$  WBC in 0.2 ml. saline. DPN reduction was measured against a control containing all the components of the mixture except glutamate. Increase in optical density from DPN reduction was linear with time for at least 60 minutes. The rate of change of optical density was shown to be proportional to enzyme concentration (and therefore to the number of WBC before disrupting) within the range studied (0 to  $8 \times 10^7$  WBC). Enzyme preparations from WBC were stable for at least 1 week when kept frozen. In the studies with WBC preparations, activity is expressed as change in optical density  $\times 10^3 / 2 \times 10^7$  WBC/30 minutes ( $\Delta OD 30$ ).

In preliminary surveys on methods for deter-

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mining GOAT, the spectrophotometric procedures of Cammarata and Cohen (3) and Green *et al.* (5) were found to be unsatisfactory for WBC because of the strong extraneous ultra-violet absorption by other WBC components. Incubation of aspartic acid and  $\alpha$ -ketoglutaric acid with WBC homogenates or plasma gave no demonstrable transamination after 90 minutes as determined by measuring glutamic acid by paper chromatography, oxalacetate formation by manometry, or analysis of the phenylhydrazones of  $\alpha$ -ketoglutaric and pyruvic acids by colorimetry (7). Karman *et al.* (6), using paper chromatography, found that measurable amounts of glutamic acid were formed by transamination of  $\alpha$ -ketoglutaric acid in plasma

RESULTS

*Glutamic acid dehydrogenase.*—Neither plasma nor disrupted RBC at levels ranging from  $1 \times 10^7$  to  $2 \times 10^8$  cells contained measurable GAD. Therefore, although the white blood cell preparations contained some RBC, the latter did not contribute to enzyme activity. The activities of WBC from nonleukemic, leukemic, and cancer patients are summarized in Table 1. The average enzyme activity was about 5 times higher in WBC from patients with leukemia or cancer than from normal individuals or patients with disorders not related to cancer and leukemia. Statistical analysis with Student's "t" test (8), used to obtain the corresponding P values, indicated that these differ-

TABLE 1  
GLUTAMIC ACID DEHYDROGENASE ACTIVITY IN WHITE BLOOD CELLS

Diagnosis or Classification	No. cases	WBC count* ( $\times 10^{-3}$ )	Mean GAD activity ( $\Delta OD_{30}$ )†	Standard deviation	P‡
<b>Leukemia:</b>					
Acute	9 (4M, 5F)§	14 -150	63 (37-137)#	34	<0.01
Chronic lymphatic	10 (7M, 3F)	3.2-377	62 (0-188)	54	<0.01
Chronic granulocytic	14 (7M, 7F)	6.9-500	61 (0-188)	53	<0.01
<b>Cancer</b>	28 (18M, 10F)	7.4-17.4	64 (0-180)	39	<0.01
<b>"Other":</b>	16 (9M, 7F)	5.3-19	14.6 (0-61)	17	0.5
Coronary disease	5 (2M, 3F)	7.0-15.0	7.8 (0-25)	9.2	
Anemia	1 (1F)	15	0		
Ulcerative colitis	2 (2M)	19, 10	3, 38		
Diabetes	2 (1M, 1F)	14, 13	0, 15		
Asthma	1 (1M)	9.4	20		
Paninusitis	1 (1M)	13.6	20		
Nephritis	1 (1M)	15.2	0		
Achondroplasia	1 (1F)	11.4	21		
Behavior problems	2 (2M)	7, 9	63, 14		
<b>Normal controls</b>	14 (8M, 6F)	5.3-7.5	17 (0-50)	15	

\* White blood counts tabulated as minimum and maximum per classification where more than two patients were studied.  
 † Change in optical density  $\times 10^4/2 \times 10^7$  WBC/30 min.  
 ‡ From Student's argument "t" for unpaired data and corresponding probability, P, that so great a difference from the mean of normal controls is due to chance (13); <0.01 indicates the difference to be highly significant; >0.05, not significant.  
 § M = males; F = females.  
 # Range.

only after 17 hours of incubation. From the other side of the equilibrium reaction, however, we found that incubation of plasma or WBC with oxalacetate and glutamate gave measurable transaminase activity after 1 hour's incubation, indicating that the equilibrium lies far in favor of aspartate rather than oxalacetate in WBC. The method finally adopted for measuring glutamic-oxalacetic acid transaminase was a modification of the manometric procedure of Ames and Elvehjem (1), with pyridoxal phosphate added as recommended by O'Kane and Gunsalus (10). Values are expressed as  $\mu$ l. CO<sub>2</sub> equivalent to the oxalacetate transaminated per  $2 \times 10^7$  WBC per hour, or 0.2 ml. plasma per hour. The transaminase determinations were performed on uncentrifuged WBC homogenates within 48 hours after drawing the blood. Preliminary experiments showed that GOAT of WBC did not change during storage at  $-5^\circ$  C. for this length of time.

ences are highly significant. No correlation between GAD activity and sex or age could be demonstrated. No consistent differences in WBC activity could be shown between the various types of leukemia or cancer. The GAD levels of selected leukemic individuals measured before and during treatment (transfusion, x-ray, Myleran or OPSPA<sup>1</sup>) were constant despite great fluctuations in the WBC count. The variation in enzyme activity within each group was high and remains unexplained.

The high GAD activity in WBC from patients with cancer was unexpected. Only a few representative cases of various cancers were examined, so that it is not possible at present to associate high white blood cell GAD activity with any specific neoplasm. However, the results suggest that an

<sup>1</sup> Oxyptamethylenediethylenethiophosphoramidate was supplied through the courtesy of Dr. Charles Heidelberger, Department of Oncology, University of Wisconsin.

alteration in WBC GAD may be involved in the cancer process.

**Glutamic-oxalacetic acid transaminase.**—In Table 2 the results of leukemic and nonleukemic WBC transaminase determinations are compared. No significant differences were observed when the various groups were compared with normal controls. The transaminase activities of the WBC appeared to be independent of the changes in the WBC count in the blood or the ratio of granulocytes to lymphocytes.

From Table 3, in which plasma transaminase values are presented, it appears that the mean plasma activity in leukemia is somewhat higher than in cancer, nonleukemic, or normal controls. However, when the data are analyzed by Student's "t" test to obtain the corresponding P values, it

activity of WBC from patients with a variety of other diseases was in general within the normal range. Particularly striking is the similarity of enzyme activity in the various types of leukemia and cancer. This suggests that those modifications of glutamic acid metabolism in WBC that exist in leukemia may also occur in cancer. Because of the wide range of activity in all classifications, the increased GAD in cancer and leukemia cannot be considered a diagnostic criterion for these diseases.

Several investigators have recently reported the presence of GOAT in blood. Marsh *et al.* (9) found the enzyme in the cellular fractions, but not in plasma. Activity was also found in the WBC-platelet fraction.<sup>2</sup> Molander,<sup>3</sup> using a spectrophotometric method, has recently demonstrated the presence of GOAT in plasma. Karman *et al.* (6)

TABLE 2  
GLUTAMIC-OXALACETIC ACID TRANSAMINASE ACTIVITY OF WHITE BLOOD CELLS

Diagnosis or Classification	No. cases	Ranges of WBC counts ( $\times 10^{-3}$ )	Mean transaminase activity ( $\mu\text{l. CO}_2$ )*	Standard deviation	P†
<b>Leukemia:</b>					
Acute	10 (4M, 6F)‡	14–150	58 (27–104)§	27	>0.9
Chronic lymphatic	7 (5M, 2F)	59–377	84 (42–132)	30	>0.9
Chronic granulocytic	12 (4M, 8F)	18–241	63 (42–111)	25	>0.9
"Other" (nonleukemia, noncancer)	17 (12M, 5F)	7.7–17.1	50 (0–159)	41	>0.9
Normal	5 (3M, 2F)	5.0–7.0	72 (43–113)	26	

\* See text for units of activity.

† P values derived from Student's "t" test for the experimental groups compared with the normal controls. If P is greater than 0.05, it is not to be considered significantly different from the control group.

‡ M = males; F = females.

§ Range.

TABLE 3  
GLUTAMIC-OXALACETIC ACID TRANSAMINASE IN PLASMA OF PATIENTS WITH LEUKEMIA AND CANCER

Classification	No. patients	Mean transaminase activity ( $\mu\text{l. CO}_2$ )*	Standard deviation*
Leukemia	34	22 (0–92)	27
Cancer	29	13 (0–95)	11
Nonleukemia, noncancer	17	9 (0–33)	10
Normal	7	12 (0–41)	16

\* See text for units of activity.

P value for comparison of leukemia or cancer with nonleukemia, noncancer was >0.05. (See reference 8.)

can be shown that these differences are not statistically significant. No marked changes in plasma or WBC transaminase were observed during chemotherapy.

#### DISCUSSION

The studies reported in this paper indicate that the average glutamic acid dehydrogenase activity of WBC from patients with leukemia and cancer is several times higher than normal. However, GAD

have also shown by paper chromatographic techniques that transaminase activity may be present in both WBC and plasma. Using manometric methods, we have confirmed these findings and extended them to a study of glutamic acid metabolism in leukemia.

Two possible pathways of glutamic acid metabolism in WBC and plasma have thus far been surveyed. GAD activity was found to be significantly higher in WBC from leukemic and cancerous patients than in WBC from controls. However, neither WBC nor plasma GOAT showed a change from normal in any of the groups studied. It is interesting to note that GAD involves oxidation-reduction mechanisms, whereas the transaminase involves essentially no gain or loss of energy. Many workers have demonstrated that in leukemia the oxidative metabolism of the WBC is altered (2). The increase in GAD may be a reflection of this change.

<sup>2</sup> L. D. Greenberg, personal communication.

<sup>3</sup> D. W. Molander, personal communication.

## SUMMARY

1. Glutamic acid dehydrogenase was estimated spectrophotometrically in white blood cells from patients with acute, chronic granulocytic, and chronic lymphatic leukemias and cancer. The average values for each of these groups were similar to one another and several times higher than in white blood cells from patients without leukemia or cancer or from "normal" blood donors. Red blood cells and plasma contained no detectable glutamic acid dehydrogenase activity.

2. Glutamic-oxalacetic acid transaminase is present in human white blood cells and plasma as determined by manometric assay. No activity was found in red blood cells.

3. No significant differences were observed in plasma transaminase activity from patients in the classifications studied.

4. White blood cell glutamic-oxalacetic acid transaminase levels were similar in the patients with various forms of leukemia, other diseases, and normal controls.

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