Oxygen Consumption of Human Leukocytes from Blood, Lymph Nodes, and Patients with Leukemia[1] (37189)

AUDREY E. EVANS AND JUDITH BLORE
(Introduced by A. S. Goldman)
The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19146

Warburg's original concept that malignant transformation results in damage to cell respiration and a secondary increase in glycolysis has been questioned following the development of more sophisticated techniques of cell fractionation and analysis. Many of the recent queries have arisen from studies of Morris rat hepatomas. Normal and malignant leukocytes are one of the most readily available human cell lines for study, but work has been hampered by failure to recognize the heterogenous nature of most leukocyte populations. Although leukocytes may have acquired, by adaption, a unique respiratory mechanism owing to their suspension in a fluid medium, a comparison of normal with leukemic cells should provide valid conclusions regarding alterations, if any, resulting from malignant transformation.

Previous studies in this laboratory of mitochondrial electron transport enzymes have shown increased activity of leukemic cells when compared to normal controls (1). In particular, succinate cytochrome C reductase, cytochrome oxidase, and the mitochondrial TPNH-DPN transhydrogenase showed a fivefold increase when the activity of mitochondria isolated from acute lymphocytic leukemic cells was compared to normal peripheral lymphocytes. Although no determinations of glycolysis were made, it was concluded from these data that respiratory activity of malignant leukocytes was increased rather than diminished.

The present study was designed for two purposes: (*1*) to determine if the increased enzyme activity, noted previously, was a reflection of increased oxygen consumption and (*2*) to obtain a more satisfactory source of pure lymphocytes for control purposes since those in the peripheral blood tend to be few in number and contaminated with neutrophils and platelets.

Materials and Methods. Normal neutrophils and blood lymphocytes were obtained from volunteer donors at The Children's Hospital of Philadelphia Blood Bank. Human lymphoid tissue was acquired following tonsillectomy, splenectomy, and partial thymectomy during cardiac surgery. Leukemic leukocytes were obtained from children with acute leukemia being cared for on the Oncology Service at Children's Hospital.

The preparation of leukocytes varied with the sources; blood lymphocytes were obtained from normal donors by phasmapheresis and whole blood centrifugation at 4000 rpm for three minutes (3500*g*). The supernatant plasma, containing the majority of platelets, was removed and the buffy coat layer containing approximately 80% or more lymphocytes was harvested. Lymphocytes were prepared from human thymus, tonsil, and spleen by mincing the tissue with scissors and suspending the preparation in tissue culture fluid (TC 199). The final suspension was filtered through nylon mesh Sephadex 400, and only preparations containing more than 70% lymphocytes were studied. Normal neutrophils and leukemic cells were obtained by sedimentation of whole blood with the addition of an equal quantity of 6% dextran in 0.9% NaCl at 37°. Anemic blood preparations sediment so rapidly on standing that a satisfactory yield was obtained without the addition of dextran. The speed of sedimentation is important since it affects the percentage of neutrophils and lymphocytes in the

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supernatant plasma. Removal of plasma following rapid sedimentation give a higher percentage of neutrophils; slower sedimentation, to allow the supernatant plasma to be clear of all red cells, yields less total leukocytes with a greater proportion of lymphocytes.

Lysis of contaminating red cells was accomplished by suspension of the packed leukocytes in 1 part TC 199 and 3 parts 0.15 *M* NH_4Cl. (Some leukemic preparations were sufficiently free of red cell contamination that their oxygen consumption was studied without lysis.) After 5 minutes equilibration in NH_4Cl, the suspended cells were centrifuged at 600*g* for 10 min, and the supernatant hemoglobin solution was decanted. A final white cell count was done. For studies of oxygen consumption, the cells were directly suspended in tris buffer, pH 7.4, while those for enzyme assay received a further wash in buffer and were stored frozen at —10°.

Mitochondria were prepared by nitrogen cavitation in an Artisan pressure homogenizer at 850 lb/in.^{2} for 20 min (2). The medium contained 0.25 *M* sucrose, 1×10^{-4} *M* $MgSO_4$, 5×10^{-3} *M* Tris, 0.13 *M* NaCl, 7.6×10^{-3} *M* Na_2HPO_4, and 1.27×10^{-2} *M* KH_2PO_4, pH 7.4 (3). Following membrane rupture, the suspension was centrifuged at 12,000*g* for 10 minutes to harvest the mitochondria. Oxygen consumption was measured by a Clark electrode in a 1.5-ml cell using Tris buffer (25 m*M* Tris, 5 m*M* MgCl, 5 m*M* KF, 20 m*M* Na_2HPO_4, 55 m*M* KCl, 45 m*M* NaCl, pH 7.4). Sodium succinate, 0.001 *M*, was used as substrate. Following homogenization of frozen packed white cells, succinic dehydrogenase activity was determined at 490 μm as the amount of formazan produced in 15 min at 37° in the presence of phenazine methosulphate (4).

Results. Oxygen consumption and succinic dehydrogenase activity of the whole cells, homogenate, and isolated mitochondria, expressed both per milligram protein and per number of cells are given in Table I. The significance of the results was calculated by the Sheffé test for simultaneous multiple comparisons using 90% confidence limits (see Table II). The consumption per cell is uniform for all types except for a slight but insignificant increase in acute myeloid leukemia (AML); 1.3 vs 2.5. The increase in oxygen consumption is more marked when related to protein content; the leukemic lymphocytes and granulocytes have significantly more activity than that of normal neutrophils and blood lymphocytes. Only preparations of mitochondria isolated from AML had significantly more oxygen consumption than those from normal cells. The wide range of results from the leukemic lymphocytes probably prevented the difference from being statistically significant.

The activty of succinic dehydrogenase calculated per cell was the same for all cell types except for an increase in myeloblasts.

TABLE IA. Oxygen Consumption.[a]

		Whole cells						Mitochondria		
		Per 1×10^7 cells			Per mg protein			Per mg protein		
Cell type	No. assayed	Mean	SD	+ Range	Mean	SD	+ Range	Mean	SD	+ Range
Blood leukocytes										
Neutrophils	5	1.3	0.5	0.6–2.0	2.1	0.7	1.4– 3.0	2.4	1.6	1.1– 4.6
Lymphocytes	18	1.5	0.8	0.5–3.2	2.7	1.2	1.1– 5.6	2.0	0.1	1.9– 2.1
Tissue lymphocytes										
Thymus	11	1.1	0.6	0.4–2.1	3.6	1.3	1.7– 6.9	8.7	7.1	2.1–19.7
Spleen	7	1.9	0.6	0.7–2.8	4.5	1.6	2.1– 6.8	5.8	0.9	4.6– 6.7
Tonsil	6	1.6	0.8	0.6–2.9	4.8	2.0	2.4– 7.5	—	—	—
Leukemic cells										
Acute lymphocytic	52	1.5	1.0	0.5–5.4	3.8	2.1	1.0– 9.0	5.1	2.7	1.2–11.4
Acute myeloid	11	2.5	1.2	1.0–5.4	5.6	3.3	2.0–12.3	14.3	7.6	3.7–25.0

[a] μmoles O_2 per minute per 1×10^7 cells or per mg protein.

TABLE IB. Succinic Dehydrogenase.[a]

Cell type	No. assayed	Whole cells						Mitochondria		
		Per 1 × 10^7 cells			Per mg protein			Per mg protein		
		Mean	SD	+ Range	Mean	SD	+ Range	Mean	SD	+ Range
Blood leukocytes										
Neutrophils	5	2.1	1.4	0.7– 3.8	4.9	1.6	2.1– 6.6	7.0	4.0	2.6– 12.1
Lymphocytes	18	2.6	1.3	1.0– 4.5	5.9	2.1	3.2– 8.7	11.4	8.4	5.2– 30.0
Tissue lymphocytes										
Thymus	11	2.1	1.1	1.3– 4.8	13.7	6.7	5.1–30.5	43.5	29.6	9.4–113.3
Spleen	7	2.0	1.2	0.4– 3.5	8.5	5.2	2.2–14.3	22.2	12.3	8.5– 38.0
Tonsil	6	0.7	0.3	0.2– 1.1	4.3	1.5	2.7– 6.6	10.8	2.8	7.1– 13.4
Leukemic cells										
Acute lymphocytic	52	3.2	2.0	0.9– 9.5	14.2	6.1	5.8–26.1	51.2	30.0	8.3–139.3
Acute myeloid	11	6.9	3.1	4.2–14.2	18.8	6.2	12.9–32.4	41.3	13.7	23.4– 66.4

[a] Succinic dehydrogenase activity is expressed as mg Fomazan/hour/mg protein or/10^7 cells.

The increase in activity of the leukemic cells was more marked when expressed per milligram protein; the difference between them and normal peripheral blood leukocytes was significant. The difference among cell types was considerable in the assays of isolated mitochondria and was seen between both leukemic cells and peripheral leukocytes and tonsil, and also between thymocytes, neutrophils, and tonsil.

Discussion. Previous studies of respiratory enzymes in leukemic cells showed increased activity of both whole cells and isolated mitochondria when compared to normal peripheral neutrophils and lymphocytes (1). The present study shows that the increased activity seen is associated with increased oxygen consumption. Two new facts emerged from this study; first, tissue lymphocytes, particularly thymocytes, have more respiratory activity than the peripheral lymphocytes, and, second, the oxygen consumption and succinic dehydrogenase activity of tissue lymphocytes is equal to that of leukemic lymphocytes. It is known that the proportions of short lived rapidly dividing lymphocytes is higher in tissue than in peripheral blood (5). Such rapid division may be associated with increased metabolic activity. Since, in this study, tissue and leukemic cells have the same range of activity, the increase of the leukemic verus blood lymphocytes may reflect turnover time rather than an alteration produced by malignant transformation. The variation of lymphocyte types found in thymus may also explain the wide range of results obtained since the thymus preparations came from children of different ages and under different "stress." The leukemic leukocytes were also harvested at different stages of disease and treatment, although all patients were in relapse. Normal myeloblasts were not studied as a control for the myeloid leukemic cells since they are not obtainable in a pure preparation.

New information resulted by expressing respiratory activity as a function of the number of cells assayed. There was a remarkable uniformity of activity when equal quantities of cells were compared. Slightly more activity was found in myeloid leukemic cells where the succinate dehydrogenase was three times higher than that of blood and tissue lymphocytes. One explanation for relatively constant activity per cell, but variations when related to protein, is less protein per cell in the more active types. The average protein values for 1 × 10^7 cells in the electrode chamber were: neutrophils, 0.6 mg; lymphocytes, 0.4 mg; tissue lymphocytes, 0.15 mg; and lymphocytic leukemia, 0.17 mg. This variation in protein can indeed explain the differences in specific activity of the whole cells, but it does not explain the increased activity found in isolated mitochondria unless one proposes that preparations of mitochondria from neutrophils and lymphocytes contain greater nonmitochondrial protein

TABLE II. Statistical Significance Comparing Cell Types.[a, b]

	Oxygen consumption/mg protein													
	Whole cells							Mitochondria						
	Polys	Lympho-cytes	Thymus	Spleen	Tonsil	ALL	AML	Polys	Lympho-cytes	Thymus	Spleen	Tonsil	ALL	AML
Polys		−	+	+	+	+	+		0	+	−	0	−	+
Lymphocytes	−		−	−	−	−	+	0		0	0	0	0	0
Thymus	+	−		−	−	−	−	+	0		−	0	−	−
Spleen	+	−	−		−	−	−	−	0	−		0	−	−
Tonsil	+	−	−	−		−	−	0	0	0	0		0	0
ALL	+	−	−	−	−		−	−	0	−	−	0		−
AML	+	+	−	−	−	−		+	0	−	−	0	−	
	Succinic dehydrogenase/mg protein													
Polys		−	+	−	−	+	+		−	+	−	−	+	+
Lymphocytes	−		−	−	−	−	+	−		−	−	−	+	+
Thymus	+	−		−	+	−	−	+	−		−	+	−	−
Spleen	−	−	−		−	−	−	−	−	−		−	−	−
Tonsil	−	−	+	−		+	+	−	−	+	−		+	+
ALL	+	−	−	−	+		−	+	+	−	−	+		−
AML	+	+	−	−	+	−		+	+	−	−	+	−	

[a] + Denotes that the difference in activity of these two cells was statistically significant within 90% confidence limits.
[b] 0 Denotes that the comparison was not made.
[c] Abbreviations, ALL, acute lymphocytic leukemic; AML, acute myeloid leukemic.

contamination. Previous work studying the purity of the mitochondrial preparations for enzyme assays using a sucrose density gradient showed that the mitochondria prepared in this way were reasonably uniform (1). The difference between the cell types was even more marked in the assays of isolated mitochondria. The increased specific activity of mitochondria isolated from tissue and leukemic cells suggests that they are indeed more active but may be fewer in number than those in neutrophils and blood lymphocytes. We believe the increased mitochondrial activity to be real. The fact that the increase in oxygen consumption is not as large as the succinic dehydrogenase may well be a result of mitochondrial damage occuring during preparation.

Summary. Oxygen consumption and succinic dehydrogenase activity were determined in whole cells and mitochondria isolated from peripheral leukocytes, tissue lymphocytes, and human leukemic cells. Tissue lymphocytes and leukemic leukocytes had higher respiratory activities than peripheral neutrophils and lymphocytes when calculated per mg protein, but not when expressed as a function of the number of cells. The fourfold increase in the activity of mitochondria isolated from tissue and leukemic cells compared to blood leukocytes suggests that the mitochondria in the former are more active but fewer in number.

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