The Action of Leucogenenol on Lymphoblastoid Cells of Normal and Neoplastic Origin[1] (35814)

F. A. H. Rice, and J. D. McCurdy

Department of Chemistry, American University, Washington, D. C. 20016

Leucogenenol, first isolated from cultures of *Penicillium gilmanii* (1) and later from bovine and human liver (2), stimulates the formation of the progenitor cells of the circulating leukocytes in both normal (3) and irradiated (4) animals. Within 24 hr after the injection of leucogenenol, granuloblasts and more immature cells are provided with higher than normal proliferation and maturation rates (5); and, consequently, injection of irradiated recipients with bone marrow from mice previously injected with leucogenenol causes an increased rate of colony formation in the spleens of the recipients (5). The maturation rate of each of the morphologically recognizable progenitors of the circulating neutrophil and erythrocyte is increased by leucogenenol (6) and neutrophils are released into the circulation to be later sequestered by such tissues as the spleen (7). The proliferation and maturation rate of lymphocytic cells is also increased (6, 7) by leucogenenol; and, consequently, there is a decrease in the time required for sublethally irradiated (8) and splenectomized (9) mice and rats to become capable of forming hemolysin antibodies.

Addition of leucogenenol to a tissue culture of lymphoblastoid cells increases the respiratory quotient (RQ) (10), increases the rate of replication, decreases the rate of oxygen consumption (10) and induces approximately 60% blastoid transformation in leukocyte cultures of human peripheral blood (11).

We wish to report that although leucogenenol increases the RQ and decreased the rate of oxygen consumption of lymphoblastoid cells that originate from the peripheral blood of normal humans, it decreases the RQ and increases the rate of oxygen consumption of lymphoblastoid cells that originate from the blood or bone marrow of humans suffering from neoplastic diseases. The RQ and rate of oxygen consumption and the effect of leucogenenol on the RQ and rate of oxygen consumption is also characteristic of the neoplastic origin of the lymphoblastoid cells.

Materials and Methods. Cultures of Cee 8068 and Papermeister 1788F were obtained through the courtesy of Dr. G. E. Moore, Roswell Park Memorial Institute, Buffalo, N. Y. Other lymphoblastoid cell lines were obtained from the Tissue Bank of the Naval Medical Research Institute, Bethesda, Maryland. In addition to Cee 8068 and Papermeister 1788F, cells of normal human origin were: Huly-47 (O'Donnell), Huly-17 (Levine) and Huly-49 (Ostrander). Cells of neoplastic origin were: Huly-6 (Burkitts lymphoma, Raji), Huly-22 (acute myelogenous leukemia, RPMI-8866), Huly-23 (multiple myeloma, IM-9), Huly-1 (chronic myelogenous leukemia) and Huly-31 (chronic myelogenous leukemia).

As previously described (8), cultures were maintained on Eagle's minimum essential medium with added fetal calf serum (20%) and buffered with bicarbonate to pH 7.2. The respiratory quotient and oxygen consumption was determined in the same medium, buffered, however, with *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (HEPES). Either 0.2 ml of pyrogen-free isotonic saline or 0.2 ml of pyrogen-free isotonic saline containing 10^{-3} μg of the calcium salt of leucogenenol was added to 3 ml of medium (approx 10^6 cells/ml) in Warburg respirometer tubes (15 ml); and the RQ and rate of oxygen consumption were determined as previously described (8).

[1] This work was partially supported by contract with the Office of Naval Research No. N00013-76-C-0275, a grant from the Life Sciences Division, Army Research Office No. DA-ARO-49-092-65-686, and grant from The Lilly Research Laboratories, Indianapolis, Indiana.

Results. Each of five strains of lymphoblastoid cells of normal human origin (Table I) showed an average RQ of 0.82 ± 0.05 and an average rate of oxygen consumption of 1.80 ± 0.1 μl of $O_2/10^6$ cells/hr. The average RQ increased to 1.13 ± 0.08 and the average rate of oxygen consumption decreased to 1.55 ± 0.1 μl of $O_2/10^6$ cells/hr when leucogenenol was added to the medium. Lymphoblastoid cells of neoplastic origin showed a significantly higher RQ ($p<0.005$ for difference) than cells of normal human origin with a range of from 1.12 ± 0.05 to 1.42 ± 0.05; and, on the addition of leucogenenol to the medium, the RQ decreased to the range 0.44 ± 0.05 to 0.82 ± 0.05. The rate of oxygen consumption of lymphoblastoid cells of neoplastic origin was significantly lower ($p<0.005$ for difference) than that of cells of normal human origin with a range of 1.13 ± 0.05 to 1.41 ± 0.05 μl of $O_2/10^6$ cells/hr. However, when leucogenenol was added to their medium, the rate of oxygen consumption increased to approximately the values found for cells of normal origin growing in a medium without added leucogenenol. The two strains of lymphoblastoid cells that originated from cases of chronic myelogenous leukemia (Huly-1 and Huly-31) were indistinguishable as regards the RQ and rate of oxygen consumption and the effect of leucogenenol on the RQ and oxygen consumption. Lymphoblastoid cells that originated from a lymphoma showed the same RQ as cells that originated from a chronic myelogenous leukemia. However, the addition of leucogenenol to the medium containing these cells caused a decrease in RQ to 0.44 ± 0.05, which is approximately one-half the corresponding RQ of cells that originated from a chronic myelogenous leukemia ($p<0.005$ for difference). The RQ of lymphoblastoid cells that originated from an acute myelogenous leukemia (Huly-22) was approximately that of cells that originated from a chronic myelogenous leukemia. However, on the addition of leucogenenol to the medium, the RQ became significantly less ($p<0.005$ for difference) than the corresponding RQ of cells that originated from a chronic myelogenous leukemia. Cells that originated from a multiple myeloma had a significantly higher RQ ($p<0.005$ for difference) of 1.42 ± 0.05 than cells that originated from a chronic myelogenous leukemia, and, on the addition of leucogenenol to the medium, showed a significantly lower RQ ($p<0.005$ for difference) of 0.68 ± 0.05 than did cells that originated from a chronic myelogenous leukemia growing in the presence of leucogenenol.

Discussion. The RQ and rate of oxygen consumption of each of five strains of lymphoblastoid cells that originated from normal humans are indistinguishable both with, and without, the addition of leucogenenol to the culture medium. It would appear therefore that, so far as the response to leucogenenol is concerned, there are no significant differences between strains of lymphoblastoid cells of normal human origin. However lymphoblastoid cells of neoplastic origin may be characterized by the RQ and rate of oxygen consumption and the magnitude of the effect of leucogenenol on the RQ and rate of oxygen consumption. The fact that leucogenenol causes an increased rate of maturation (5, 6) of the progenitors of the peripheral leukocytes and also increases blast formation of human peripheral leukocytes in tissue culture (11) suggests the possibility that leucogenenol acts on an enzyme system that in tissue culture results in a change in RQ and oxygen consumption, while in the animal the same system is associated with the maturation or transformation of blood cells. The uniformity of action of leucogenenol on lymphoblastoid cells of normal human origin compared to the variable and characteristic action of leucogenenol on lymphoblastoid cells of neoplastic origin suggests the possibility that malfunctioning of the system that is affected by leucogenenol is responsible for neoplastic blood diseases.

Summary. In tissue culture leucogenenol increases the RQ and decreases the oxygen consumption of lymphoblastoid cells that originate from normal humans, while it decreases the RQ and increases the oxygen consumption of lymphoblastoid cells that originate from humans suffering neoplastic blood diseases. Strains of lymphoblastoid cells that originate from normal humans are indistin-

TABLE I. The Effect on the Respiratory Quotient (RQ)[a] and Oxygen Consumption[a] of Adding 10^{-3} μg of Leucogenenol to Lymphoblastoid Cells of Normal and Neoplastic Origin Growing in a Medium[b] buffered with HEPES.[c]

Cell type	RQ		O_2 consumption (μl of $O_2/10^6$ cells/hr)	
	Without leucogenenol	With leucogenenol	Without leucogenenol	With leucogenenol
Normal origin				
Cee 8068	0.85 ± 0.05	1.12 ± 0.03	1.90 ± 0.07	1.61 ± 0.04
Papermeister 1788F	0.80 ± 0.02	1.01 ± 0.03	1.80 ± 0.08	1.63 ± 0.12
Huly-47	0.82 ± 0.05	1.16 ± 0.05	1.76 ± 0.05	1.43 ± 0.10
Huly-17	0.82 ± 0.05	1.20 ± 0.05	1.83 ± 0.05	1.65 ± 0.05
Huly-49	0.81 ± 0.05	1.17 ± 0.05	1.72 ± 0.06	1.44 ± 0.10
Av	0.82 ± 0.05	1.13 ± 0.08	1.80 ± 0.10	1.55 ± 0.1
Neoplastic origin				
Huly-6 (lymphoma)	1.24 ± 0.05	0.44 ± 0.05	1.41 ± 0.05	1.81 ± 0.05
Huly-22 (acute myelogenous leukemia)	1.10 ± 0.05	0.56 ± 0.05	1.33 ± 0.05	1.64 ± 0.05
Huly-23 (multiple myeloma)	1.42 ± 0.05	0.68 ± 0.05	1.25 ± 0.05	1.70 ± 0.05
Huly-1 (chronic myelogenous leukemia)	1.16 ± 0.05	0.80 ± 0.05	1.13 ± 0.05	1.73 ± 0.05
Huly-31 (chronic myelogenous leukemia)	1.21 ± 0.05	0.82 ± 0.05	1.23 ± 0.05	1.76 ± 0.05

[a] Standard deviations calculated from results on a minimum of 6 experiments.

[b] Approximately 3 ml of Eagle's minimum essential medium with added fetal calf serum (20%) containing approximately 10^6 cells/ml.

[c] *N*-2-Hydroxyethylpiperazine-*N'*-ethanesulfonic acid obtained from Calbiochem., Los Angeles, Calif.

guishable so far as the effect of leucogenenol on them is concerned. Lymphoblastoid cells that originate from neoplastic tissues, however, show a RQ and rate of oxygen consumption and the effect of leucogenenol on the RQ and rate of oxygen consumption that is characteristic of the neoplastic origin of the lymphoblastoid cells. It is suggested that leucogenenol affects those enzyme systems that are associated with the transformation of cells and perhaps malfunction of the same systems is associated with neoplastic diseases.

The authors thank Dr. K. W. Sell, Naval Medical Research Institute, Bethesda, Maryland, for the lymphoblastoid cells.

1. Rice, F. A. H., Proc. Soc. Exp. Biol. Med. **123**, 189 (1966).

2. Rice, F. A. H., and Shaikh, B., Biochem. J. **116**, 709 (1970).

3. Rice, F. A. H., and Darden, J. H., J. Infec. Dis. **118**, 76 (1968).

4. Rice, F. A. H., Lepick, J., and Darden, J. H., Radiat Res. **36**, 144 (1968).

5. Fossati, G. C., Fumarola, D., Cerra, E., and Cavalieri, E., Riv. Emoter. Immunoematol. **16**, 91 (1969).

6. Rice, F. A. H., McCurdy, J. D., and Aziz, K., Proc. Soc. Exp. Biol. Med. **136**, 56 (1971).

7. Rice, F. A. H., Connolly, J., Aziz, K., and McCurdy, J. D., J. Infec. Dis. **123**, 117 (1971).

8. Rice, F. A. H., Lepick, J., and Hepner, P., Radiat. Res. **42**, 164 (1970).

9. Rice, F. A. H., and Ciaverra, R., Proc. Soc. Exp. Biol. Med. **137**, 567 (1971).

10. Rice, F. A. H., Blum, M. L., and Rene, A. A., Proc. Soc. Exp. Biol. Med. **135**, 623 (1970).

11. Fumarola, D., De Rinaldis, P., and Maruccio, L., G. Batteriol. Virol. Immunol. **18**, 11 (1970).

Received Apr. 29, 1971. P.S.E.B.M., 1971, Vol. 137.