and decrease van der Waals interactions between hydrocarbon chains. Trehalose, by contrast, is capable of expanding even fully condensed monolayers (*24*) and therefore must associate with the PC by a different mechanism. Infrared spectroscopic studies of dry PC-trehalose (Fig. 2) and PC-glycerol (Fig. 3) mixtures are consistent with this explanation. The data for glycerol (Fig. 3) show that in place of the complex interactions seen in the trehalose-PC mixtures, the only change in the PC spectrum was in the band at 1246 cm^{-1}, which was shifted to 1240 cm^{-1} with increasing concentration of glycerol. The band broadening seen in the trehalose-PC spectrum was missing, as were the changes in OH stretching bands in the carbohydrate.

These data show that while the ability of a molecule to depress the melting temperature of a dry phospholipid is probably important in preserving biomembrane structure in the dry state, it is not in itself sufficient; glycerol depresses T_c, but its mode of interaction with the phospholipid, probably intercalation between the head groups, carries with it deleterious side effects such as fusion. As a result, glycerol does not preserve dry membranes. Such side effects are not found with trehalose, probably because its interactions with phospholipids involve hydrogen bonding between OH groups in the trehalose and the phosphate head group. These hydrogen bonds may replace the same or similar hydrogen bonds between the lipid and water that occur in bulk water. The relative specificity of the bonding between trehalose and phosphate head groups may therefore be an important factor in the ability of this molecule to replace water around the head group of a phospholipid and thereby to stabilize dry membranes.

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27. DPPC (purissimum) was purchased from Fluka and used without further purification. Samples were prepared by dissolving 10 to 20 mg of DPPC (weighed to the nearest 0.01 mg) and an appropriate amount of trehalose in 2 ml of methanol to which 1 ml of benzene was added after the samples were fully dissolved. The solution was frozen in liquid nitrogen and lyophilized. Subsamples of the dry preparations were weighed and were sealed in DSC pans, and calorimetric curves were recorded with a Perkin-Elmer DSC-2 calorimeter. At least three heating endotherms were recorded for each sample to confirm stability of the sample. The data were converted to digital form with a Hewlett-Packard graphics computer-plotter, normalized to a standard weight of lipid, and replotted in the form presented in Fig. 1. T_c was measured according to (*25*). Enthalpies (reported as calories per gram of lipid) were calculated from the areas of the heating endotherms, using published values for the main transition of hydrated DPPC as a standard (*15, 26*).
28. Subsamples (approximately 1 mg) from the same preparation (*27*) were weighed to the nearest 0.01 mg, ground with 100 mg of KBr, and pressed into disks. Spectra were obtained with a Perkin-Elmer IR681 spectrometer linked to a Perkin-Elmer 3500 data station. The data, obtained in digital form, were corrected for small differences in sample weights by introduction of the appropriate factor on the data station, smoothed by the Savitsky-Golay function, and replotted as shown in Fig. 2.
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Lymphokine Production by Cultured Human T Cells Transformed by Human T-Cell Leukemia-Lymphoma Virus-I

Abstract. *Cell-free conditioned media from human T cells transformed by human T-cell leukemia-lymphoma virus (HTLV-I) were tested for the production of soluble biologically active factors, including several known lymphokines. The cell lines used were established from patients with T-cell leukemia-lymphoma and from human umbilical cord blood and bone marrow leukocytes transformed by HTLV-I in vitro. All of the cell lines liberated constitutively one or more of the 12 biological activities assayed. These included macrophage migration inhibitory factor (MIF), leukocyte migration inhibitory factor (LIF), leukocyte migration enhancing factor (MEF), macrophage activating factor (MAF), differentiation inducing factor (DIF), colony stimulating factor (CSF), eosinophil growth and maturation activity (eos. GMA), fibroblast activating factor (FAF), γ-interferon and, in rare instances, T-cell growth factor (TCGF). Some cell lines produced interleukin 3 (IL-3), platelet-derived growth factor (PDGF), or B-cell growth factors (BCGF). Such cells should prove useful for the production of lymphokines and as sources of specific messenger RNA's for their genetic cloning.*

T cells play an important role in cell-mediated immunity both as effector cells and as modulators of cell proliferation and function. Many of these T-cell functions are mediated by soluble, biologically active molecules called lymphokines (*1*). These include among others: macrophage migration inhibitory factor (MIF) (*2*), leukocyte migration inhibitory factor (LIF) (*2*), leukocyte migration enhancing factor (MEF) (*2*), neutrophil migration-inhibitory factor (NIF-T) (*3*), macrophage activating factor (MAF) (*4*), differentiation inducing factor (DIF) (*5*), colony stimulatig factor (CSF) (*6*), eosinophil growth and maturation activity (eos. GMA) (*6*), interleukin 3 (IL-3) (*7*), fibroblast activating factor (FAF) (*8*), chemotactic factors (*1*), lymphotoxins (*9*), T-cell growth factor (TCGF) (*10*), B-cell growth factor (BCGF) (*11*), interferon (*12*), and other activities involved with helper and suppressor T-cell function. Some of these activities are well characterized while most essentially remain phenomenological observations not associated with distinct molecules. Progress in biological and biochemical characterization of many of these activities has been hampered by difficulties in ob-

taining sufficient amounts of active material. The most common sources have been mitogen-stimulated fresh peripheral blood mononuclear cells, some established leukemic T-cell lines (*13*), and hybrid cell lines established by fusion of activated fresh T cells with established T-cell lines (*14*). We and others have also described the production of some biological activities by T-cell lines established in cultures from patients positive for human T-cell leukemia virus (HTLV-I). For example, Gootenberg *et al.* (*15*) described the constitutive production of low levels of TCGF by the cell line HUT 102, and Le *et al.* (*16*) reported that a subclone of this cell line could liberate α- and γ-interferon. Also, cell line MO, established from a patient with a variant of hairy cell leukemia, was reported to liberate γ-interferon and CSF (*17*). MO was subsequently found to produce a subtype of HTLV-I, HTLV-II (*18*). In this report we extend these observations and describe a new approach to the routine production of several human lymphokines based on the development of T-cell lines immortalized in vitro by infection with HTLV-I (*19*).

The T-cell lines were established from patients with HTLV-positive T-cell malignancies (*15*) or from human umbilical cord blood and bone marrow T cells transformed in vitro by HTLV-I (*19*). All of them (Table 1), with the exception of UK, grew without exogenous TCGF. As previosly described (*19*), the HTLV-I–infected cell lines grew with a population doubling time of 40 to 60 hours and reached a saturation density of $1^{-3} \times 10^6$ cells per milliliter. Most of the cell lines [established adult T-cell leukemia-lymphoma donors and HTLV-I–transformed cord blood and adult bone marrow leukocytes] were reactive with monoclonal antibodies Leu 1 (T-cell specific), OKT 11 (E-rosette receptor specific, data not shown).

Table 1. Characteristics of HTLV-I–transformed human T lymphocytes. T cells, initially established in suspension culture from peripheral blood of adult patients with T-cell leukemia-lymphoma, were lethally irradiated and used as a source of virus to infect umbilical cord blood T cells and adult bone marrow T cells by cocultivation procedure. Virus donor cells are represented by the initials of the donor leukemia patient. Other cell lines used are labeled C for cord blood or B for bone marrow followed by sample number in the numerator and the cell line used as a source of HTLV in the denominator. All cell lines shown except UK grew independently of added TCGF. Cell lines C43/UK, C63/CR FII, B2/CR FII, and B1/MJ were nonproductively infected by HTLV.

Culture designation	Surface phenotype*					HTLV protein† p19,p24
	OKT3	OKT4/ Leu 3a	OKT8/ Leu 2a	OKT10	Leu 7	
Cultured leukemic T cells						
CR FII	0	100	0	0	0	90
MJ	40	90	0	0	4	90
UK	100	100	0	0	0	50
Transformed umbilical cord blood T cells						
C9/MJ-1	0	100	0	0	0	80
C9/MJ-2	18	90	0	0	0	85
C10/UK-1	0	80	0	0	0	80
C10/UK-2	45	35	4	10	0	90
CS/MJ	80	70	30	0	0	75
C91/PL	20	50	0	0	0	60
C43/UK	15	90	0	0	0	0
C63/CR FII	20	100	0	0	0	0
Transformed adult bone marrow T cells						
B1/MJ	0	0	0	0	0	0
B2a/MJ	40	20	2	8	4	80
B2b/MJ	30	12	4	10	4	65
B2/UK	80	3	45	4	4	80
B2/CR FII	0	90	0	0	0	0
B3a/MJ	10	15	7	5	0	70
B3b/MJ	5	100	16	4	0	30
B8b/C10UK	8	10	0	0	0	90
B9a/C10UK	23	5	40	5	3	45
B9/C10MJ	0	0	0	0	3	80
B9b/C10UK	15	0	36	10	4	90
B10/C10UK	35	5	45	40	6	8
B11/C10UK	40	8	38	10	5	25

*Cell type specific monoclonal antibodies were used to characterize live cells by an indirect immunofluorescence procedure (*19*). Numbers indicate the percentage of positive cells reacting with the indicated antibody. In addition, all cell cultures were negative for surface-bound immunoglobulins, Epstein-Barr virus nuclear antigen, OKT6 and OKM1, and positive for HLA-DR antigens (780 percent) and TCGF receptors (750 percent). †Antibodies to purified HTLV structural proteins p24 and p19 were used in indirect immunofluorescence assays on fixed cells (*19*). Numbers indicated the percentage of positive cells.

As shown in Table 2, T lymphocytes from leukemic donors and transformed cord blood were usually positive for the OKT 4/Leu 3a (helper/inducer) phenotype and negative for OKT 8/Leu 2a (cytotoxic/suppressor phenotype) (*19*). Some HTLV-I–transformed bone marrow T cells, however, were OKT 8/Leu 2a positive and OKT4/Leu 3a negative, and some expressed neither set of antigens. The HTLV-I–transformed lymphocytes usually had an atypical, granular-globular staining pattern for nonspecific esterase and acid phosphatase as has been reported for HTLV-I–transformed T lymphocytes. These cells lacked myeloid cytochemical markers, for example, myeloperoxidase and chloroacetate esterase, and B-lymphocyte markers, for example, surface immunoglobulin and Epstein-Barr virus nuclear antigens (*19*). All cell cultures contained HTLV-I–specific nucleic acids, expressed at least low levels of viral proteins (*19*), and with the exception of C43/UK, C63/CR FII, B1/MJ, and B2/CR FII released intact HTLV. The four nonproducer cell lines (Table 1) described here did not release HTLV detectable by banding in sucrose, electron microscopic observation, or by transmission to susceptible cells. However, these cells contained at least one copy per cell of HTLV proviral DNA and transcribed viral RNA, but levels of viral structural proteins were 10- to 100-fold lower than in virus-producing cells (*19, 20*).

Fluid from cell cultures was collected 24 to 48 hours after a complete media change, and was clarified by low-speed centrifugation and filtration (0.22 μm). Serial dilutions were tested for biological activity by established procedures. All media and reagents were screened for endotoxin activity and only endotoxin-free materials were tested. As positive controls we used conditioned media from normal human peripheral blood mononuclear leukocytes stimulated with mitogen. In addition to the HTLV-transformed T-cell lines listed in Table 2, fluids from other sources were tested and found negative for the activities described in this report. For example, the media and fetal calf serum used for growing all the cell cultures, conditioned media from a "pre–T-cell" line established from a leukemic patient [Molt 4 (*21*)], B-cell lines from normal donors (*22*), and long-term myeloid and monocytic cell cultures (*23*) were tested. Some of the monocytic cells did liberate lymphocyte activating factor (LAF), prostaglandin E, lysozyme, and CSF (data not shown).

MIF, defined as the ability to inhibit

the migration of fresh human macrophages, was tested by a microcapillary procedure (*2, 24, 25*). The area (in square centimeters) of migration of treated versus untreated adult peripheral blood macrophages (*25*) was expressed as a migration index. Cell culture fluids from normal human mononuclear leukocytes treated with purified protein derivative (PPD) were used as positive controls for MIF. Compared to untreated macrophages with a migration index ranging from 0.80 to 1.20, macrophages treated with conditioned media from several cell lines had a reduced migration index, that is, ≤ 0.80 (Table 2).

LIF, defined as a factor involved in restricting granulocyte migration from the site of an inflammatory response, and MEF, a factor that enhances this migration, were assayed in the same way as MIF (*24*). The responder cells were mature granulocytes prepared from adult peripheral blood by differential centrifugation in Ficoll-Hypaque. Fluids from several cell lines either restricted leukocyte migration (migration index of ≤ 0.80, defined as LIF activity) or stimulated leukocyte migration (migration index of ≥ 1.20, or less defined as MEF activity).

MAF, a factor that nonspecifically activates macrophages for extracellular killing, was tested by a procedure described by Fidler *et al.* (*26*). Monocyte-macrophages, prepared as for the MIF assay, were first "activated" by incubation for 24 hours either with conditioned medium from the HTLV-I–transformed T-cell lines or with MAF-positive supernatant fluids collected from concanavalin A–treated human peripheral blood monocytes. Both resulted in a 40 to 50 percent level of cytotoxicity. The activated cells were then added to radiolabeled melanoma cell line A376 and, after an additional 72 hours of incubation, the cytolytic effect was determined by measuring the residual radioactivity in the melanoma cells. MAF activity is expressed as the percentage of cytotoxicity compared to that obtained with nonactivated cells. Approximately one-half of the cell lines tested released a significant amount (≧ 20 percent) of activity (Table 2).

DIF, a factor released by activated lymphocytes that induces the morphological and functional maturation of human myeloid leukemia cells (*5*), was assayed by incubation of the promyelocytic cell line HL-60 (*27*) for 4 days in the presence of 10 n*M* retinoic acid (*5*) and increasing concentrations of test cell fluids. Differentiation of HL-60 was determined by the increased number of cells able to reduce nitroblue tetrazolium or to phagocytize yeast (*28*). Media from all cell lines assayed induced HL60 maturation and contained DIF ranging from 500 to 2000 U/ml (Table 2).

CSF, a family of factors able to support the growth and maturation of monocytes and granulocytes in semisolid medium (*6, 23, 29*), was assayed by incubating fresh bone marrow leukocytes in 0.3 percent agarose containing various concentrations of test fluids. Many of the cell lines released CSF at levels comparable to or higher than positive control samples from a human trophoblast cell line (*30*) (Table 2). The type of cells forming the colonies were granulocytic or monocytic as determined by Wright-Giemsa staining of colonies picked from the agarose plates or by staining in situ (*31*) (data not shown).

Eos. GMA, a factor that supports the maturation and cell division of fresh eosinophils in vitro (*6*), was assayed by incubation of conditioned media from T-cell lines with fresh bone marrow or cord blood mononuclear leukocytes. In positive samples the usually small population of recognizable eosinophils was rapidly expanded by a combination of limited cell replication and maturation of immature cells (Table 2). The resulting cell population was > 90 percent eosino-

Table 2. Lymphokines released by HTLV-I–transformed human T lymphocytes. The cultures were as described in the legend for Table 1. Serial dilutions of conditioned media, collected from cell lines 24 to 48 hours after a change of medium, were tested for the indicated activities and compared to standard positive control samples as described in the text. For MIF, a migration index of ≤ 0.8 indicates activity. Supernatant fluids from PPD-treated normal human mononuclear leukocytes were used as positive controls. For LIF and MEF, a migration index of ≤ 0.08 represents LIF and ≥ 1.2 MEF activity. For MAF, an activity of ≧ 20 percent was considered significant. Fluids from concanavalin A–treated human monocytes were used as a positive control with 40 to 50 percent cytotoxicity. For DIF, + indicates 500 to 1000 U/ml; ++, 1000 to 10,000 U/ml; +++, > 10,000 U/ml; and −, < 500 U/ml. For CSF, values represent the number of colonies (≧ 50 cells per colony) per 10^5 fresh bone marrow cells seeded in 0.33 percent agarose. Eos. GMA, assessed as the ability of conditioned media to induce and support cell division and maturation of eosinophils from fresh bone marrow or umbilical cord blood. Symbols: +, maturation and one cell doubling; ++, maturation and two to four cell doublings in 2 weeks; +++, maturation and more than four doublings in 2 weeks; −, no growth and negligible maturation. For FAF, + indicates that labeled thymidine incorporation in resting human fibroblast cells was about two times greater than with medium only; −, indicates an activity less than twice that of background. For interferon, the data are expressed as international units of interferon activity compared to a 5000 U/ml standard.

Culture designation	Biological activities							
	MIF (migration index)	LIF/MEF (migration index)	MAF (% cytotoxicity)	DIF (U/ml)	CSA (colonies/10^5 cells)	Eos. GMA	FAF	Interferon −PHA/+PHA (U/ml)
Cultured leukemic T cells								
CR FII	0.22	1.40	< 20	+	150	+++	+	14/0
MJ		0.75	< 20	+	50	+++	+	0/0
UK		0.62	< 20		5	+++	−	
Transformed umbilical cord blood T cells								
C9/MJ-1	0.55	0.70	< 20	++	40	+	+	0/0
C9/MJ-2		1.25	< 20	++	60		+	0/0
C10/UK-1			< 20	+++	125	+++	+	0/0
C10/MJ-2	0.62	1.05	31	+++	0	+++	+	0/0
C5/MJ	0.51	1.20	21	+	50		−	0/0
C91/PL	0.34	1.30	< 20	+	40		+	0
C43/UK	0.31	1.40	35	++	70	+++	−	0/0
C63/CR FII	0.67	1.70	36	+++	40	+++	+	0/0
Transformed adult bone marrow T cells								
B1/MJ	0.67	1.00	< 20	+++	25	+	+	0/600
B2a/MJ	0.38	0.60	26	+	60	+	+	0/0
B2b/MJ		0.55	72	++	60	+	+	524/3900
B2/UK			54	+	75	+	+	
B2/CR FII			< 20	+++	30	+++	+	832/416
B3a/MJ	0.51		73	+	50	+	+	39/0
B3b/MJ				+	0			0/0
B8b/C10UK		0.75	67	++	65	++	+	39/0
B9a/C10UK	0.40	1.70	< 20	++	75	−	−	212/316
B9/C10MJ			< 20	+	100			0/0
B9b/C10UK		0.70			56	+	−	4400/4062
B10/C10UK	0.56	0.70	< 20	+	95	+	+	1400/4054
B11/C10UK			< 20	+	60		+	274/2400

as confirmed by Luxol fast blue staining and electron microscopic examination for characteristic granuoles (data not shown).

FAF, a product of human peripheral blood lymphocytes that stimulates the active proliferation of quiescent fibroblasts (*8*), was assayed by testing the ability to stimulate [^{3}H]thymidine uptake in resting cells from fibroblast cell line CCD1SK in an assay described by Schmidt *et al.* (*32*). Conditioned media from many of the cell lines were positive in this assay (Table 2). This biological activity is similar to that described for platelet-derived growth factor (PDGF) (*33*). By means of immunopurification techniques with hyperimmune sera against purified human PDGF, cell lysates from all of the HTLV-I–transformed cell lines tested contained material related to PDGF (*34*).

Interferon, which belongs to a family of glycoproteins liberated by lymphocytes, exhibits a potent effect on virus replication and cell growth. Interferon was assayed by a plaque-reduction technique in which we used vesticular stomatitis virus (VSV) with monolayers of the human cell line WISH as a target (*35*). Test samples collected from the cell line were induced, or not induced, by exposure to phytohemagglutinin-P (PHA-P; 5 μg/ml for 24 hours) and incubated with target cells for 24 hours before infection with VSV. Interferon activity, measured by comparison to an international standard (5000 U/ml), was not detected in fluids from induced or uninduced cells established from T-cell leukemia-lymphoma patients or from HTLV-transformed cord blood T cells (Table 2). However, several HTLV-transformed bone marrow cell lines produced substantial levels of interferon, often without PHA-P induction. This activity was destroyed (≥ 80 percent) by treatment at *p*H 2 for 24 hours, suggesting that the activity was caused by γ-interferon (*12, 35*) (data not shown). Whether or not all HTLV-I-positive cells liberate γ-interferon needs further investigation. All cell lines examined, including some found negative for interferon production in the biological assay, transcribe detectable levels of polyadenylate-containing RNA recognized by cloned γ-interferon complementary DNA assayed by Northern blot procedures (*36*).

TCGF (also known as interleukin-2) is liberated by activated T lymphocytes and participates in the regulation of T-cell growth (*10, 37*). Cell culture fluids concentrated 20 times and eluates from intact T cells treated at low *p*H (*15*) were examined for TCGF activity by a thymidine uptake procedure (*37*). TCGF was detected at low levels in conditioned media or bound to the cell membrane of some cell lines established from adult T-cell leukemia-lymphoma patients, for example, CR and MJ. However, with the exception of membrane-bound TCGF detected on C9/MJ-1, no activity was associated with any of the other HTLV-I–transformed cord blood or bone marrow T cells (data not shown).

In addition to the factors described in Table 2, tests for other biological activities were performed. For example, IL-3, an activity liberated by mitogen-activated lymphocytes which supports the growth of immature hemotopoietic cells in vitro, was produced by many of the cell lines (*38*). Also BCGF, assayed by a thymidine uptake procedure (*39*), was produced by some cell lines (data not shown).

Several biologically active molecules produced by monocytic cells [lymphocyte activating factor (LAF), prostaglandin E, and lysozyme] were also assayed. Prostaglandin E and lysozyme, assayed by radioimmunoassay and a radial diffusion procedure (*23*) were not detected. However, a low level of LAF, assayed with fresh thymocytes from C3H/HEJ mice as indicator cells (*40*), was detected in some of the cell lines tested (*41*).

The biological activities described herein were detected in unconcentrated tissue culture fluids from most of the HTLV-positive T-cell lines studied and, in contrast to nonactivated T cells from normal donors (*1*), they were constitutively produced in most instances. No attempt was made to optimize the expression or recovery of the activities and it is likely that higher levels of activity could be obtained with proper manipulation. The role HTLV plays in the induction of lymphokine synthesis is unclear. It is not known, for example, whether the lymphokines described would normally be produced by the subsets of T cells infected by HTLV-I or whether infection by HTLV-I induces their synthesis. It is clear that a productive HTLV infection is not necessary since transformed cells not producing virus, for example C43/UK, C63/CR FII, B2/CR FII, and B1/MJ, also produce the lymphokines. These HTLV-transformed nonproducer T-cell lines will be of particular value in the study of these lymphokines since they reduce the potential biohazard caused by the presence of HTLV.

The ability to establish immortalized T-cell lines by infection with HTLV should prove useful for studies of the biological activities detected, some of which are otherwise difficult to obtain in large quantities. The HTLV-transformed T cells should also provide sources of messenger RNA for the genetic cloning of the biological factors.

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Hypoxic Coordinate Regulation of Mitochondrial Enzymes in Mammalian Cells

Abstract. *The effect of hypoxic exposure on various mitochondrial enzymes and on cell mitochondrial genomic content was studied in two types of mammalian cells. Hypoxia depressed the activity of six enzymes to the same degree. The kinetics of depression and of recovery during reexposure to normoxia were statistically similar for three marker enzymes. Despite the global and symmetrical decrease in enzyme activities, mitochondrial DNA remained constant. This suggests either symmetrical loss of mitochondrial enzymes from all mitochondria or complete loss of enzymes from a subpopulation of mitochondria with retention of an intact mitochondrial genome.*

It is generally accepted that there are two major mechanisms for the regulation of overall mitochondrial composition. One of these regulatory mechanisms determines the number of mitochondria per cell. This function is largely related to the rate of mitochondrial replication, which must involve synthesis of mitochondrial DNA (mtDNA) (*1*). Since the various cell types presumably achieve a steady state with respect to mitochondrial number, the rate of mitochondrial degradation must be regulated as well. The second of the major mechanisms regulates individual proteins making up mitochondria. This is accomplished largely through nucleus-directed protein synthesis on cytoplasmic ribosomes, but there is an independent mitochondrial transcription-translation system that regulates the biosynthesis of special mitochondrial components (*2*). Because various mitochondrial components have variable turnover times (*3*), variable degrees of nuclear and mitochondrial control (*4*), and variable specific genomic expression (*5*), individual proteins appear to be individually regulated.

We have obtained evidence that there is another form of regulation. During incubation under hypoxic conditions, various mitochondrial enzymes show a generalized decrease in activity. The magnitudes of these decreases are statistically similar for all enzymes, as are the changes with time in enzyme activity for three index enzymes. This suggests a coordinate regulation of mitochondrial function. Measurements of mtDNA content show that there is no significant change during hypoxic incubation. Hence, coordinate depression of enzyme levels is not simply the result of a reduction in mitochondrial number produced by alterations in the rate of mitochondrial replication or degradation.

The decreases in mitochondrial enzyme activity with an unchanged mtDNA content must mean that there is a general inhibitor of mitochondrial enzyme activity, or that all mitochondria symmetrically and reversibly lose a substantial segment of mitochondrial enzyme content, or that a subpopulation of mitochondria can reversibly revert to an elementary form that lacks mitochondrial enzymes but contains mtDNA.

Two different cell types were studied. Mouse lung macrophages, obtained by lung mincing (*6*) were cultured in a system in which the cells do not replicate. Rat skeletal muscle L8 cells (*7*) in early passage were cultured in a system in which replication does occur. The results were essentially the same for both cell types, indicating that cell replication is not a major factor in coordinate regulation. Each cell type was cultured under normoxic (PO_2, ~ 120 torr; PCO_2, ~ 40 torr) or hypoxic (PO_2, 15 to 25 torr; PCO_2 ~ 40 torr) conditions.

In the first series of studies, enzyme activities of six separate enzymes were measured after 96 hours of incubation. Four enzymes located on the mitochondrial matrix were studied. Three of these—citrate synthetase, nicotinamide-adenine dinucleotide (NAD^+)-dependent isocitrate dehydrogenase, and malate dehydrogenase—participate in the tricarboxylic acid cycle, and glutamate dehydrogenase functions as a link between carbohydrate and amino acid metabolism. Also studied were succinate dehydrogenase, an inner mitochondrial component of the Krebs cycle, and cytochrome aa_3, a component of the inner membrane mitochondrial electron transport chain.

We measured enzyme activities by

Table 1. A comparison of mitochondrial enzyme activities under normoxic (PO_2, 120 torr) and hypoxic (PO_2, 15 to 25 torr) conditions after 96 hours of incubation. All enzyme activities under hypoxic conditions were significantly less ($P < 0.05$ except where noted) than under normoxic conditions ($N = 6$ for all studies except where noted). Analysis of variance revealed that in each cell type the degree of depression of mitochondrial activity for each enzyme was statistically the same with the exception of cytochrome aa_3 in L8 rat skeletal muscle cells. Succinate dehydrogenase activities in mouse lung macrophages were too low to be measured. Values are expressed as the mean ± standard deviation (S.D.).

Enzyme	Enzyme activity (μmole/min-mg)		Percent change
	Normoxia	Hypoxia	
L8 rat skeletal muscle cells			
Citrate synthetase	0.150 ± 0.021	0.105 ± 0.012	29.8 ± 6.9
NAD-isocitrate dehydrogenase	0.014 ± 0.002	0.009 ± 0.002	36.0 ± 13.0
Mitochondrial malate dehydrogenase	3.43 ± 0.41	2.70 ± 0.41	22.7 ± 8.0
Succinate dehydrogenase	0.036 ± 0.006	0.027 ± 0.006	26.3 ± 8.9
Cytochrome aa_3	0.038 ± 0.015	0.021 ± 0.008	42.8 ± 9.4
Glutamate dehydrogenase	0.019 ± 0.004	0.014 ± 0.002	28.2 ± 5.4
Mouse lung macrophages			
Citrate synthetase	0.219 ± 0.047	0.111 ± 0.050	49.4 ± 24.5
NAD-isocitrate dehydrogenase	0.012 ± 0.004	0.006 ± 0.002	49.2 ± 12.7
Mitochondrial malate dehydrogenase*	0.897 ± 0.211	0.580 ± 0.104	39.7 ± 21.1
Cytochrome aa_3	0.198 ± 0.060	0.108 ± 0.045	45.4 ± 15.0
Glutamate dehydrogenase	0.175 ± 0.032	0.102 ± 0.038	48.0 ± 16.5

*For malate dehydrogenase in mouse lung macrophages, $N = 3$, $P < 0.1$.