The Effect of Dissolved Oxygen Partial Pressure on Growth, Metabolism and Immunoglobulin Production in a Permanent Human Lymphocyte Cell Line Culture[1] (36092)

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The growth of microorganisms and cells *in vitro* under controlled environmental conditions was a goal of many studies and research projects in the past. One of the environmental factors which was investigated was the effect of dissolved oxygen partial pressure (pO_2).

The effect of pO_2 on growth of bacteria and molds and on their metabolism has been investigated thoroughly (1).

Less work has been done on this subject using animal cell cultures. Cooper *et al.* (2) investigated the effects of different oxygen tensions on the growth of ERK cells in continuous culture. They found that the cell growth rate was dependent on the oxygen level in the gas phase. They also observed that high levels (25–30%) of oxygen in the gas phase were cytocidal (3).

Harris *et al.* (4) developed the Meta-Stat that included a gas monitor which automatically monitored and controlled the pH and gas content of the liquid in a suspension cell culture system. Later they investigated the environmental conditions for growth of L-929 mouse cells (5). Kilburn and Webb (6, 7) developed a 3-liter culture vessel for the growth of animal cells in suspension under well-controlled liquid pO_2. They reported that maximum cell populations were obtained when the pO_2 was controlled at values within a range of 40–100 mm Hg.

Andersen *et al.* (8) studied the effects of pO_2 on DNA synthesis and on the number and structure of mitochondria (9) in human lymphocytes stimulated with phytohemagglutinin.

The aim of this work was to investigate the effects of pO_2 on growth, glucose metabolism, and immunoglobulin (Ig) production in a permanent human lymphocyte cell line in suspended culture. Immunoglobulin was chosen as a representative of biologically active products produced by lymphocyte cell lines.

Materials and Methods. Cell Line. A permanent hematopoietic (lymphocyte) cell line designated Roswell Park Memorial Institute (RPMI) cell line 7430 was derived from the peripheral blood of an individual with osteogenic sarcoma. This cell line produced IgM of Kappa type.

Growth conditions. Medium. For both seed and experimental cultures, RPMI medium 1701 supplemented with 4% (V/V) heat-inactivated fetal bovine serum was used. This medium is similar to RPMI medium 1640 (10) except for the following changes: Calcium nitrate and magnesium sulfate were reduced to 50 mg/liter, each. The following ingredients were added (mg/liter): oxalic acid, 150; sodium pyruvate, 50; sodium carboxymethyl cellulose, 1000 (Edifas B-50, 15 cps, Imperial Chemical Industries, Ltd., Stevenston, Ayrshire, Scotland); *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 7000; and insulin, 200 units/liter. The pH of the medium was 6.9–7.1.

Growing vessels. Seed and experimental cultures were grown in 14-liter fermentors (MicroFerm, New Brunswick Scientific Co., New Brunswick, NJ). The fermentors contained 4 baffles and one impeller. The impeller was mounted at the 3-liter level height. Cultures of cells were kept in the growth phase, to initiate experiments, by daily feeding with fresh medium. Experimental cultures were started with 0.5 to 1.0 liters of stock

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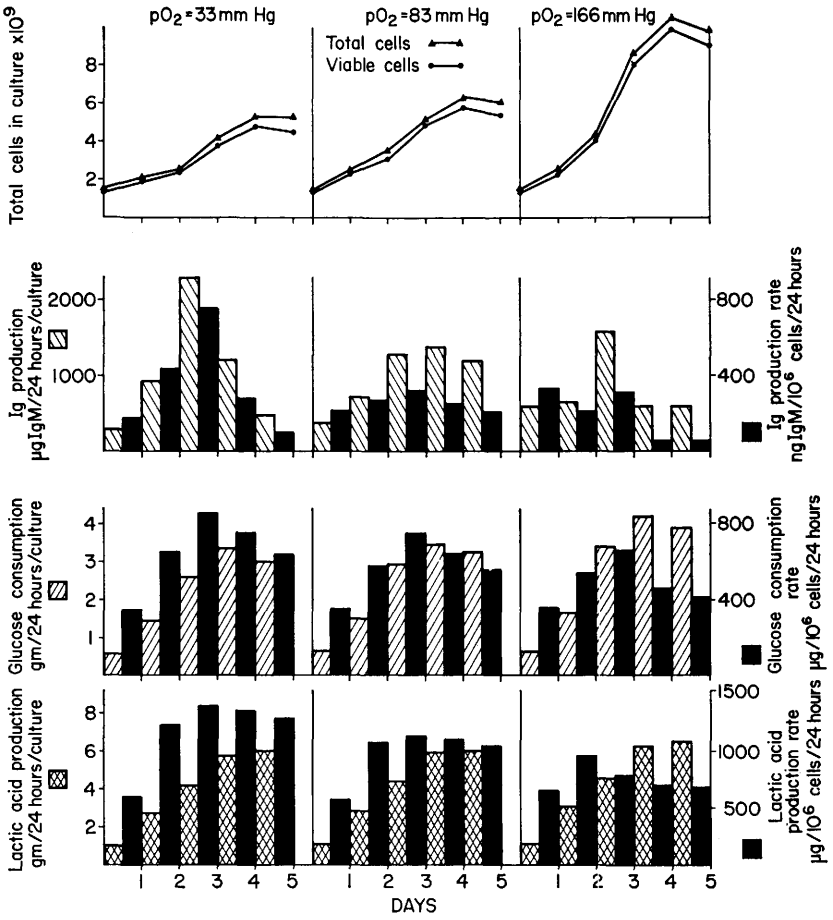

Fig. 1. Effect of different pO_2 on growth, glucose consumption, lactic acid and immunoglobulin production by RPMI cell line 7430.

cultures and the culture volume was increased to 6.0 liters with fresh medium. Initial cell concentration was adjusted to 2.0 to 2.5 $\times$ 10^5 viable cells/ml. Cultures were incubated at 37°. Experiments were run for 5 days, unless otherwise indicated.

Oxygen measurement and control. The pO_2 in the culture liquid phase was measured by a steam-sterilizable oxygen electrode (cat. No. M-1016-0200) and controlled using a Model DO-60 dissolved oxygen controller (both from New Brunswick Scientific Co.). The controller was modified so high–low control about the set point could be accomplished. Generally the pO_2 was controlled by top aeration of pure nitrogen or compressed air. In experiments where the pO_2 was zero, only pure nitrogen was allowed to flow. In experiments where the pO_2 was higher than its value in the air, the pO_2 was controlled by flowing pure nitrogen and a mixture of 40% oxygen and 60% nitrogen. The agitation speed of the impeller was always kept at 100 rpm.

Laboratory investigations. Every 24 hr, a sample of the culture was taken for investigation. Cell counts, glucose and lactic acid content were determined as described previously (11). The glucose level was adjusted daily to 2 mg/ml and the pH to 6.9–7.1 by adding 2% NaOH.

The concentration of human Ig in the culture supernatant was determined by a mi-

TABLE I.

pO_2 (mm Hg)	Total Ig production (μg of IgM for 5 days)	Molar ratio (av) lactic acid production : glucose consumption
33	5190	1.80
83	4944	1.72
166	4008	1.58

croassay method developed by N. Tanigaki and Y. Yagi. The method is based on the competitive inhibition of the binding of ^{125}I-labeled IgM to monospecific goat anti-μ antibody by the test specimen. IgM–anti-μ antibody complexes are precipitated by the addition of rabbit antiserum against goat immunoglobulins. The Ig concentration in the test specimen is determined from a reference binding curve obtained by incubating constant amounts of anti-μ antibody first with increasing amounts of a standard IgM and then with constant amounts of ^{125}I-labeled IgM. Details of the method will be published elsewhere.

Results. Effect of pO_2 on cell growth. The results given in Fig. 1 and Table I show that the highest cell yield was attained at pO_2 of 166 mm or approximately 100% air saturation with O_2. In cultures grown at a lower pO_2 (83 and 33 mm Hg), the growth rate was slower. High cell viability was observed in all the above-mentioned studies. No growth was observed when the pO_2 was kept near zero. No growth was achieved at the high pO_2 = 250 mm Hg (oxygen 100% air saturation, pO_2 = 166 mm Hg).

In another series of experiments using cultures which had been exposed for 3 days at different pO_2 levels and exhibited little or no growth, the pO_2 was gradually increased to 166 mm. (This pO_2 gave the best results.) Slight recovery of growth rate was observed in cultures which were grown at pO_2 = 33 and 83 mm Hg (Fig. 2). On the other hand, no cell recovery was achieved in the cultures exposed to extremely low or high pO_2 (0 or 250 mm Hg).

Effect of pO_2 on immunoglobulin production. The Ig production was investigated in those cultures in which cell growth was observed. The findings given in Fig. 1 indicated

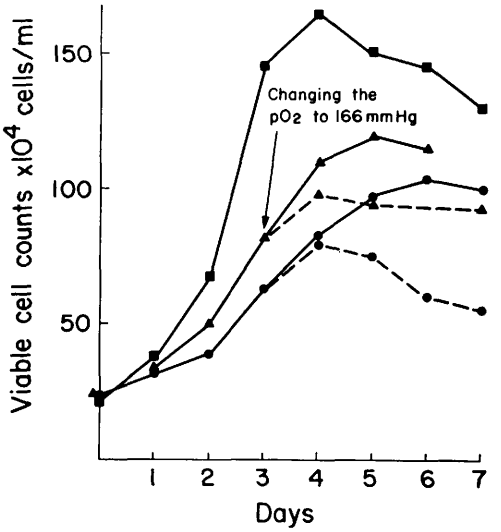

FIG. 2. Effect of increasing the pO_2 on cell recovery from hypoxia. (●) 3 days growth in pO_2 = 33 mm Hg then changed to pO_2 = 166 mm Hg; (▲) 3 days growth in pO_2 = 83 mm Hg then changed to pO_2 = 166 mm Hg; (■) control culture: growth in pO_2 = 166 mm Hg; (- - -) control culture at same pO_2 as the first 3 days.

that best production of Ig was at a high pO_2 on the first day. Thereafter, larger amounts of Ig were produced as the liquid phase pO_2 was decreased from 166 to 33 mm Hg. The production of Ig was most active while the cells were proliferating (12).

Effect of pO_2 on glucose consumption and lactic acid production. The total glucose consumption and lactic acid production were higher in cultures grown at a higher pO_2. After the first day, the calculated rates per million cells of glucose consumption, lactic acid production, and the molar ratio of lactic acid produced to glucose consumption were the lowest in cultures grown in the highest pO_2 (Fig. 1).

Discussion. There is widespread interest in studies of human lymphocyte cell lines and a need for large quantities of cells (13). Roswell Park Memorial Institute has a new pilot plant for growing lymphocyte or other mammalian cell lines (14). Cell production in these facilities could reach an estimated peak of 1 kg of wet cells/day. The propagation of lymphocyte cell lines is done under controlled conditions (temperature, pH, gas

phase CO_2, etc.) to achieve the highest cell yield per time and per medium volume. One of the environmental factors which had not been studied was the pO_2.

The oxygen consumption by animal cells is lower than that of microorganisms. This means that the liquid phase pO_2 in cell culture can be controlled by top aeration and not by sparging. Aeration of most cell cultures by sparging caused damage to the cells (7, 15). Top aeration with nitrogen or nitrogen–air mixtures will keep the pO_2 at a selected level with an accuracy of $\pm 3.0\%$ oxygen air saturation.

The lymphocytes were grown in RPMI medium 1701 which contains HEPES buffer. This buffer eliminated the rapid pH changes in the culture which might have affected the results. This problem is well known in media buffered with bicarbonate. Preliminary experiments indicated that a higher cell yield was achieved when lymphocyte cell lines were grown in this medium.

Our findings on cell growth showed that the highest cell yield was achieved at a pO_2 of 166 mm Hg, *i.e.*, 100% air oxygen saturation. Increasing the oxygen concentration to $pO_2 = 250$ mm Hg was cytocidal and no growth was attained when $pO_2 = 0$, *i.e.*, in anaerobic conditions.

The effect of pO_2 on the growth of different animal cells varied according to several authors. In work carried out with LS-mouse and with ERK-rabbit cells, it was found (7) or estimated (2) that the liquid phase oxygen level should be about 70 mm Hg (much less than in the air) for faster growth. In contrast, results obtained by Shaw and Pace (16) indicated that the L line cells (mouse fibroblasts) proliferated best when exposed to a gas mixture containing 20% oxygen. These same findings were attained by Danes and Leinfelder (17) in chick heart cultures; these results are similar to our results.

Several investigators reported that certain other animal cell lines are very susceptible to damage by oxygen at concentrations higher than that in the air (3, 18, 19). These results with different mammalian and chick cells are similar to our findings with lymphocytes. On the other hand, studies with chick skin and heart cells indicated that these cells grew actively in pure oxygen (20). Lieberman and Ove (21) suggested that one cause of oxygen toxicity may be an accumulation of peroxides in the medium.

While Shaw and Pace (16) showed that mouse fibroblast cells could recover from the effects of hypoxia and hyperoxia, we failed to recover cultured human lymphocytes from similar states.

These findings indicate that a generalization concerning the effects of oxygen on various mammalian cells cannot be made. Effects of varying oxygen concentrations should be studied with each type of cells cultured. Monitoring and continuously controlling the pO_2 in the culture fluid phase rather than in the culture gas phase is advantageous.

The effects of pO_2 on Ig production and on cell growth were different. Low Ig production was attained at high pO_2 levels while the maximum Ig production was observed at a low pO_2 which did not favor cell growth. The way that oxygen influences the regulation of Ig production is unknown.

It is interesting that the amount of oxygen present in human arterial plasma would equilibrate with a gas mixture containing about 12% oxygen (22), *i.e.*, 95 mm Hg.

The total glucose consumption and lactic acid production seemed to be higher in cultures grown in high pO_2 and reflected the increased cell growth. However, when calculated, lower rates of glucose consumption or lactic acid production were found for these cultures except on the first day. The fact that a lower glucose consumption rate was observed at a higher pO_2 may be explained as "Pasteur effect." Self *et al.* (23) studied the influence of pO_2 on the level of various enzymes in mouse LS cells. They found that the effect of low pO_2 was reflected in a decreased level of some of the enzymes involved in terminal respiration. Low pO_2 caused an increase in the enzymes involving the glycolysis and the hexose monophosphate pathways. A similar explanation may possibly be applicable to cultured human lymphocytes.

These findings may be helpful in the propagation of permanent lymphocyte cell lines to attain large amounts of cells or for max-

imum production of Ig. The pO_2 levels for achieving maximum yields are different for cell and Ig production.

Summary. The effect of liquid phase pO_2 (concentration equivalent of dissolved oxygen) on the growth of human lymphocyte cell line cultures was investigated. The pO_2 was monitored and controlled continuously and automatically. A maximum cell yield was obtained with a pO_2 of 166 mm Hg and lower cell yields were observed with lower pO_2. No cell growth occurred in cultures where the pO_2 was zero or higher than the normal 100% oxygen saturation. Immunoglobulin production was pO_2-dependent. Higher yields and synthetic rates of immunoglobulin were observed at low pO_2 (33 mm Hg).

Glucose consumption and lactic acid production rates increased as the pO_2 decreased.

Maintenance of the optimal pO_2 is desirable for the cultivation of large quantities of lymphocytes and for increased immunoglobulin yields.

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