Effect of high dilution quinones on O_2 uptake by peripheral blood lymphocytes: a polarigraphic study

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Summary

The aim of this study is to investigate whether aberrant peripheral blood lymphocytes can be corrected by the addition of a highly diluted quinone to the culture medium. The oxygen uptake of both normal and aberrant lymphocytes is examined before and after the quinone is added. The results will indicate whether the quinone is able to increase the oxygen uptake rate of aberrant lymphocytes, thereby returning them to normal metabolism.

Introduction

Lymphocytes are white blood cells produced in bone marrow. Two types exist: B-cells (cells which achieve immuno-competence in the bone marrow); and T-cells (immuno-competence achieved in the thymus). Lymphocytes respond to antigens found in the body by producing antibodies (B-cells) or lymphokines (T- and B-cells). These control the adaptive immune response by secondary action on the participating cells or kill virally-infected host cells. Lymphocytes have molecules on their surface called receptors which bind to specific antigens in order to stimulate the immune response.

Previous studies have suggested that cancer could be a metabolic disorder (Warburg, 1930, 1956; Szent-Gyorgyi, 1979) correctable using high redox potential quinones. Further studies have shown that highly diluted quinones can be effective in stimulating immune responsiveness in mice (Hidvegi, Raso et al., 1999), and lowered metastasis with quinone treatment is reported in recent clinical trials of cancer patients (Jakab, Shoenfeld et al. 2003).

This investigation examines whether the addition of parabenzoquinone in high dilution is able to correct cultured aberrant lymphocyte metabolism, known to show less oxygen uptake than normal cells.

It is hypothesized that the addition of this highly diluted quinone to a solution of aberrant lymphocytes will have a beneficial effect on the blood cells by boosting their immune response and returning them to normal metabolism. This reaction would result in an increase in the level of oxygen uptake within the solution which can be monitored polarigraphically.

Materials and Methods

The main apparatus in this experiment is the Digital Model 10 Oxygen Electrode (Rank Brothers Ltd, Cambridge, UK) used to measure the amount of dissolved O_2 in a solution. The temperature in the chamber of the oxygen electrode is controlled by a DC10-P5 water bath (ThermoHaake, UK) and kept at a constant 37˚C throughout the experiment. The data from the electrode is logged using a Delta Logger (Delta-T Devices, Cambridge, UK) which has also been programmed to take continuous measurements of room temperature, water bath temperature, and the temperature at the base of the water chamber on the electrode where the stirring mechanism is located, using separate SKTS 200/U temperature probes (Skye Instruments Ltd, Powys, UK). Humidity and room temperature are logged using a SKH 2011 probe (Skye Instruments Ltd, Powys, UK). The level of light is recorded using an LX-01 light meter (Lutron). The Delta Logger is programmed and controlled by a PC using Ls2Win 1.0 software, collecting readings from each of these probes and devices every second, then calculates and records the mean average over each 5 sec. period.

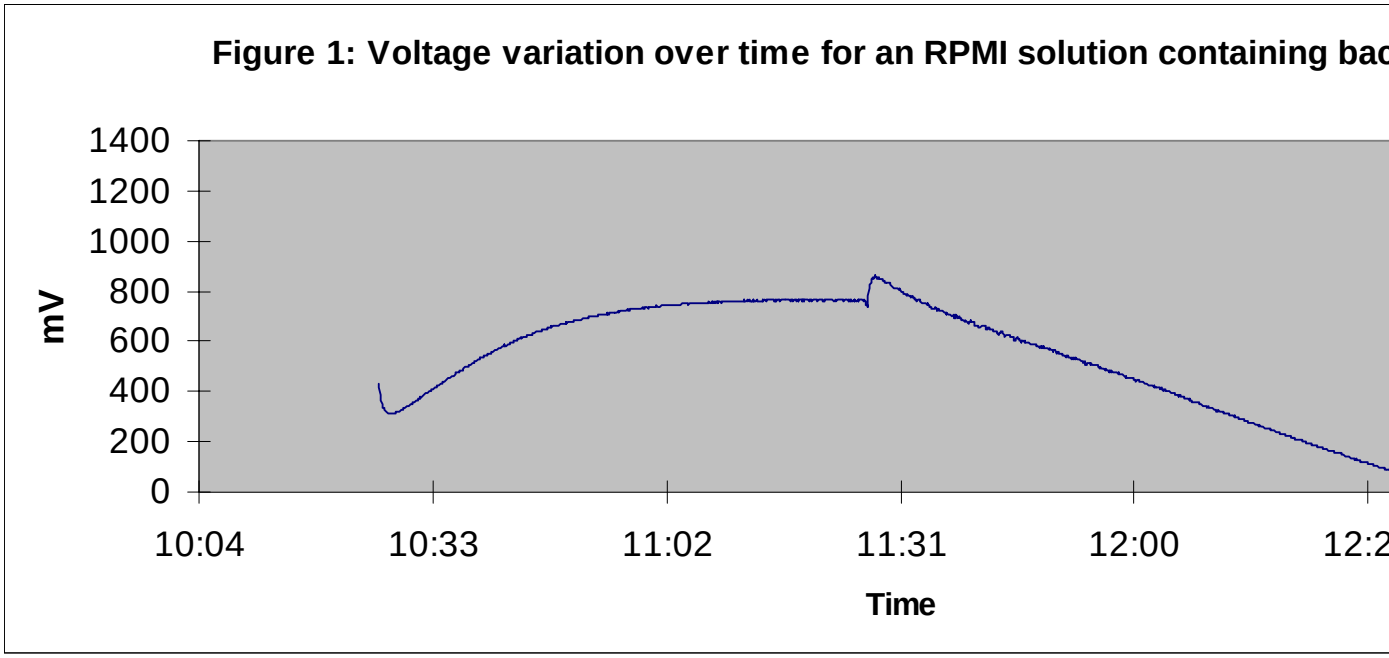
Peripheral blood lymphocytes are isolated in a nutrient medium (RPMI 1640) and standardized. Initially readings are taken for about an hour, to allow any temperature effects to settle down and to ascertain the rate of O_2 uptake by the cells. A 0.5ml solution containing 10^{-6} diluted parabenzoquinone is then added to the test chamber and readings are monitored for further hour to examine the effect of the quinone on the rate of O_2 uptake in the solution.

The electrode uses a magnetic stirrer in the chamber to circulate the solution, so it is possible that the magnetic field from this may have an adverse effect on the experiment. To mitigate this, a thin layer of polythene is inserted into the chamber to separate the stirrer from the reaction.

Initial tests to validate the equipment and procedures involved have been carried out using *E. coli* bacteria cultivated in RPMI-1640 solution. The bacteria have during the three month pretest period digested all but a trace of the glucose in the solution, which makes them ideal to use as a model. O_2 uptake from the bacteria can be measured before and after provision of additional glucose.

Results

The work is ongoing at present; however a sample of the results collected is given below.



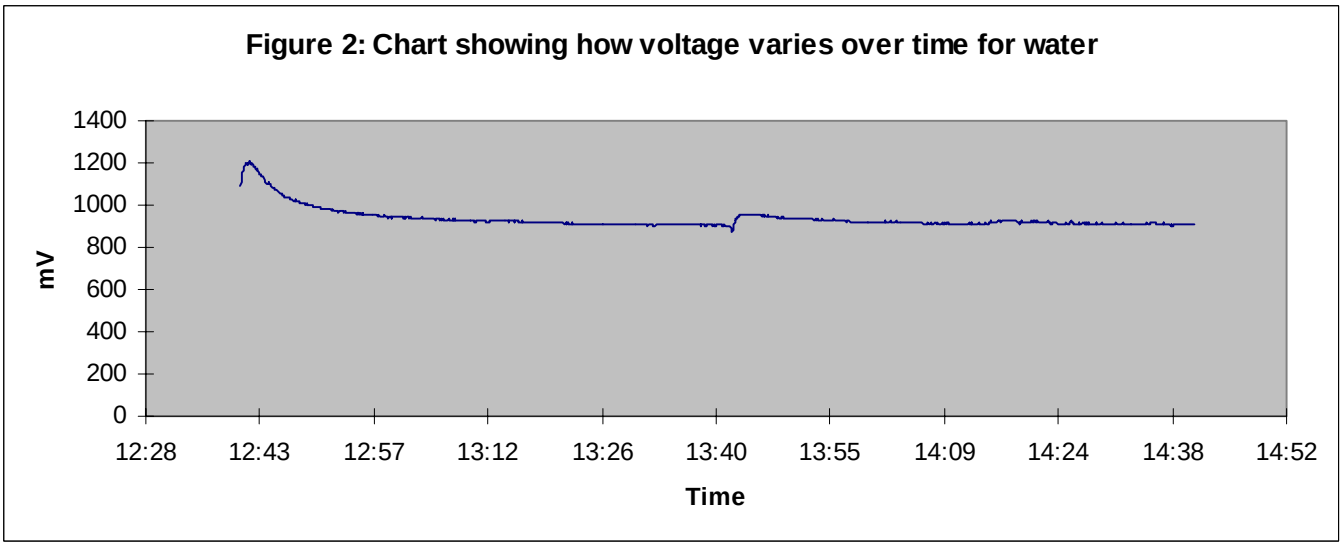
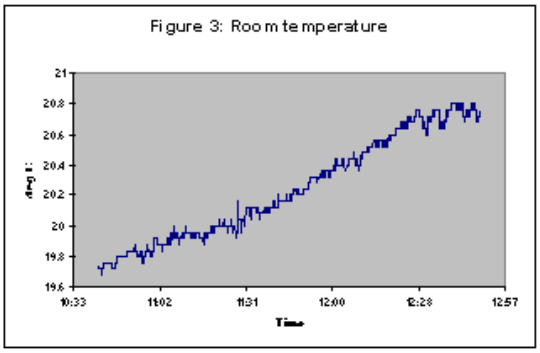


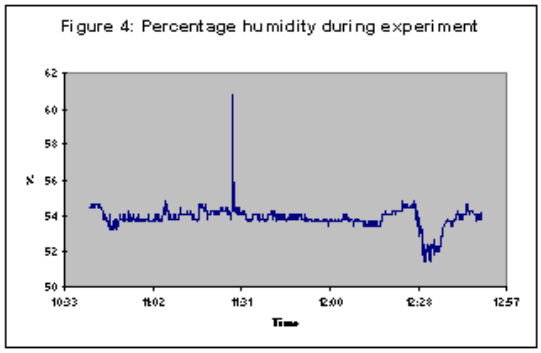
Figure 1 shows how the voltage collected from the oxygen electrode, which is proportional to the partial pressure of oxygen in the medium under test varies over time. Used in Fig.1 is 2ml of an RPMI-1640 solution containing *E. coli* bacteria, the bacteria had consumed all but a

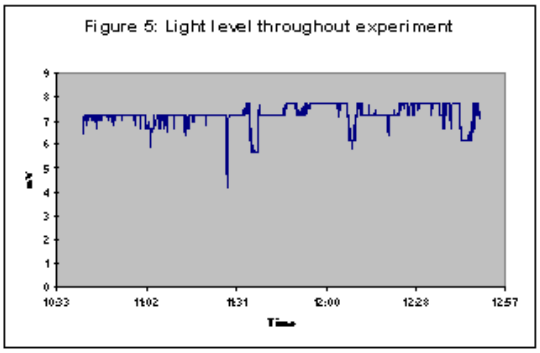
trace amount of glucose in the original container. Half way through the 2 hrs approx. experiment, at 11:27am 0.5ml of a 0.05% glucose solution was added to feed the bacteria. The subsequent initial increase is due to the dissolved oxygen in the glucose solution. The voltage then drops steadily due to the bacteria feeding on the glucose, since their increased metabolism leads to a fall in the amount of oxygen present in the test sample.

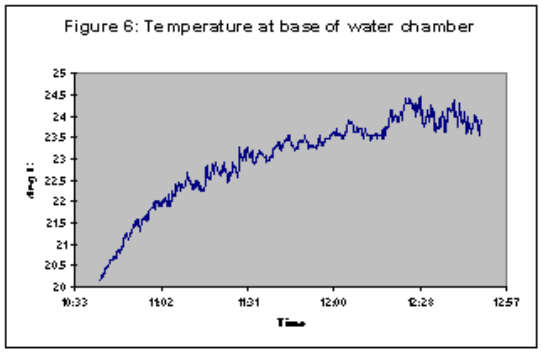
Figure 2 shows the results from a similar experiment performed as a control. The initial test solution is 2ml of distilled water. As before, half way through at 1:43pm, 0.5ml of a 0.05% glucose solution is added. It is clear that both before and after the glucose was added the level of dissolved O_2 remains the same.

As mentioned above, the apparatus was designed to allow continuous measurement of not only the level of dissolved oxygen, but also room temperature, humidity, the light level, water bath temperature, and the temperature at the base of the water chamber on the electrode. Examples of this data can be seen in the figures below.









All of this data is logged over the course of the experiment. If there is a significant difference when measuring the dissolved O_2 in the test solutions, then temperature, light, or humidity environmental effects can be excluded.

Discussion

Under normal conditions, a healthy organic cell is able to supply its own energy by producing molecules of adenosine triphosphate (ATP). This is done by a three stage process involving firstly glycolysis, then the Krebs-Szent-Gyorgyi or tricarboxylic acid (TCA) cycle, and finally oxidative phosphorylation (ox-phos pathway). We believe that in cancer cells, it is possible that this metabolic process is interrupted, and the final stage of the energy production in the cell is blocked by a carcinogen, either viral or chemical. Albert Szent-Gyorgyi also believed that high redox potential quinones (which contain active para and ortho carbonyl groups) or serial carbonyls such as glyoxal may be able to correct this fault in the metabolic pathway and return the cell to normal (Szent-Gyorgyi, 1979, 1983).

The study is based on the fact that healthy cells make use of the oxygen in their culture medium as a final electron acceptor in the ox-phos pathway. One of the features of cancer

cells however is that they do not so readily uptake dissolved O_2. The rate at which the dissolved O_2 is used up can be examined using the procedure described above, to evaluate the normality of the cells.

The aim of this study is to identify if highly diluted parabenzoquinone can correct the metabolic function of aberrant lymphocytes. The introduction of parabenzoquinone, a high redox potential quinone into separate culture mediums of normal and aberrant lymphocytes will allow us to examine if the addition of the quinone affects the rate of oxygen uptake in the samples. An increase in oxygen consumption may indicate that the cancer cells are returning to normal and re-establishing their original metabolic pathway.

The initial results from experiments using *E. coli* confirm the sensitivity of the system; since there is a clear distinction between test samples with and without bacteria when adding glucose.

Acknowledgement

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