IONIC AND OSMOTIC EFFECTS ON CELL VOLUME AND OXYGEN CONSUMPTION OF RAT BONE MARROW CELLS*

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Introduction

Two problems frequently encountered in metabolic studies are osmotic balance (Troshin 1966) and ion effect (Bacq 1965). The ions used in this study were the common buffering ions bicarbonate and phosphate, and a substituted taurine, *N*-(2-hydroxyethyl)piperazine-*N'*-2'-ethanesulphonic acid (HEPES), recently synthesized by Good *et al.* (1966). It was felt that a comparison of changes in cell volume and oxygen consumption induced by alterations in various media constituents might disclose some of the effects that cell volume and ion content (MacLeod and Rhoads 1939; Pages and Delaunay 1945; Evans and Bird 1949) have on cellular metabolism.

Materials and Methods

Male Holtzman rats, 42 ± 2 days old, were used in all experiments. Animals were killed by a sharp blow to the base of the skull and the two femora and tibiae removed. The bones were split lengthwise and the marrow teased apart in a modified Tyrode's balanced salt solution (the standard medium used in this work). The composition of this and other media used is given in the following tabulation:

Medium	Composition
Tyrode's	8·0 g NaCl, 0·2 g KCl, 0·2 g $CaCl_2$, 0·1 g $MgCl_2.6H_2O$, 0·5 g $NaH_2PO_4.H_2O$, 1·0 g $NaHCO_3$, 1·0 g glucose, in 1 litre distilled deionized water; pH adjusted to 7·35; osmolarity adjusted to 310 m-osmoles/l with NaCl.
OS 200	Tyrode's solution, but with osmolarity adjusted to 200 m-osmoles/l by decreasing the amount of NaCl.
OS 400	Tyrode's solution, but with osmolarity adjusted to 400 m-osmoles/l by increasing the amount of NaCl.
NC	Tyrode's solution, but with bicarbonate replaced by 1·0 g HEPES.
NP	Tyrode's solution, but with phosphate replaced by 0·5 g HEPES.
HPS	Tyrode's solution, but with bicarbonate and phosphate replaced by 1·5 g HEPES.
BC	Tyrode's solution containing only half the normal amount of bicarbonate and phosphate (0·5 g $NaHCO_3$, 0·25 g $NaH_2PO_4.H_2O$).
HPC	Tyrode's solution containing 0.75 g HEPES, 0·5 g $NaHCO_3$, and 0·25 g $NaH_2PO_4.H_2O$ in place of the original amounts of bicarbonate and phosphate.

Cell suspensions were obtained by passing the bone marrow successively through two 100-mesh stainless steel screens. Determination of cell counts and cell size were made on aliquots of the marrow cell suspension using a Coulter counter model B coupled to a model J Coulter chart recorder.

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After the sampling of the bone marrow cell suspension for counting and sizing, an aliquot containing 8×10^7 cells was diluted with the desired media to 5 ml and placed in the sample chamber of a model 53 Biological Oxygen Monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio) for measurements of oxygen consumption. This was done by first calibrating the oxygen electrode against distilled water saturated with air at 37°C and ambient barometric pressure. By using this value as the full-scale calibration point of the YSI amplifier unit, the oxygen content of the suspending medium could be determined relative to that of water at the beginning of each experiment. The Bunsen coefficient at 37°C was used to calculate oxygen concentration in terms of microlitres per millilitre of water. Since the Tyrode's solution and serum contained salts, the percentage of oxygen dissolved in these media was always less than the 100% obtained for the full-scale calibration in distilled deionized water. The percentage of oxygen dissolved in Tyrode's solution varied from 96 to 99% and in serum from 85 to 95% relative to that in water.

The oxygen uptake of the electrode itself was determined in distilled deionized water, Tyrode's solution, and serum. Oxygen uptake varied from 0 to 3% of full scale in these liquids. For this reason it was decided to check electrode oxygen uptake before each experiment in the medium to be utilized. The value obtained from such electrode checks was subtracted from experimental values to give net oxygen consumption of the marrow cell suspension. Time lapse from the death of an animal to the start of oxygen-uptake determinations with the oxygen electrode did not exceed 30 min. Changes in pO_2 were plotted directly by means of a VOM-7 Bausch and Lomb recorder.

Results

A typical size distribution curve of rat bone marrow cells in Tyrode's solution is shown in Figure 1. It can be seen that the cells of the population comprise three

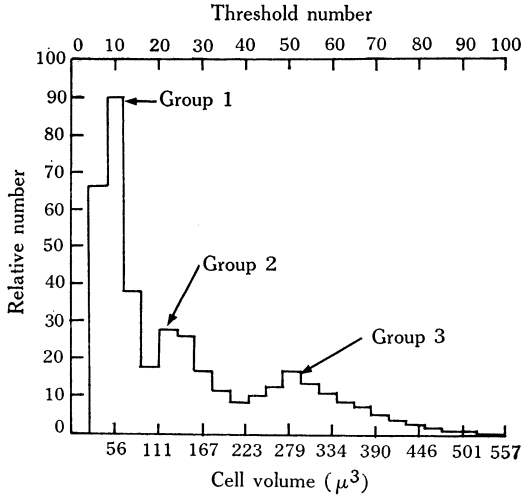

Fig. 1.—Histogram showing typical size distribution of rat bone marrow cells in Tyrode's medium. The bone marrow cell population is classifiable into three major groups on the basis of the frequency distribution of cell sizes.

distinct groups. Because the graph out registered by the Coulter counter is a frequency distribution of cell sizes, the peaks shown do not represent a pure population of any one cell type (Mattern, Brackett, and Olson 1957). Differential counts and ocular micrometer measurements of cells from prepared slides showed that the bone marrow cell population consisted of the following groups: group 1 with mature erythrocytes and a small number of small lymphocytes; group 2 with nucleated erythroid cells and lymphocytes; and group 3 with mature granulocytes and a few large blast cell forms.

Figure 2 shows the effect of different media on cell volume. Volume determinations were made on aliquots of the marrow cell suspension at the beginning and at the termination of each experiment. It can be seen that group 1 cells are least affected by alterations in composition of the suspending medium. Group 2 cells exhibit some fluctuations in volume, while group 3 cells showed the greatest response to variations in medium constituents. Figure 2 also shows that cell volume is most

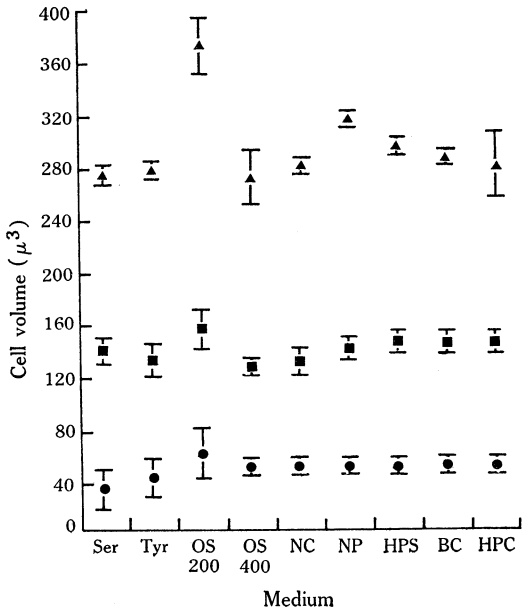

Fig. 2.—Mean variation of cell volume of groups 1, 2, and 3 bone marrow cells incubated in different media. Ser, serum; Tyr, Tyrode's solution. See text for composition of other media. ▲ Group 3 cells. ■ Group 2 cells. ● Group 1 cells. Each plot is the mean value of 10 determinations.

affected by lowering of the osmolarity of the standard medium to 200 m-osmoles/l, and by omission of inorganic phosphate. Both of these conditions resulted in a marked volume increase of cells in the group 3 category. Increase in cell size also occurred

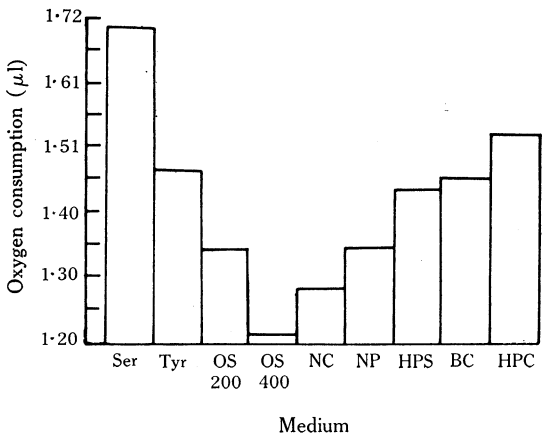

Fig. 3.—Oxygen consumption (μl/10^7 cells/20 min) of rat bone marrow cells incubated in different media. Total cell concentration used was 8×10^7 cells in 5 ml of the various media. Each bar of the histogram is the mean value of 10 experiments. Standard errors of the mean values for each medium are: serum (Ser), 0·18; Tyrode's solution (Tyr), 0·24; OS 200, 0·19; OS 400, 0·12; NC, 0·18; NP, 0·17; HPS, 0·34; BC, 0·22; HPC, 0·08.

in groups 1 and 2 when the osmolarity of the standard medium was lowered. There was no significant change in the volume of cells in groups 1 and 2 after incubation of the bone marrow in the NP medium. Both of these conditions (i.e. lowered os-

molarity and absence of inorganic phosphate) produced a comparable reduction in the oxygen uptake by bone marrow cells (Fig. 3). In both cases, this decrease in oxygen consumption reached the lower significance level ($P<0\cdot05$) within an incubation time of 20 min.

Increasing the osmolarity of the standard medium to a level of 400 m-osmoles/l resulted in wide variations in the size of group 3 cells and a marked reduction in oxygen uptake. The latter was significant ($P<0\cdot01$) after a 20-min incubation period. Bone marrow cells incubated in the NC medium showed little change in cell size. The rate of oxygen consumption of these cells was significantly reduced ($P<0\cdot01$) in this medium. No significant changes in either cell volume or oxygen uptake was observed in medium BC, although the volume of group 3 cells increased slightly. A slight increase in the size of cells in group 3 resulted when HPS medium was used. The rate of oxygen consumption of bone marrow cell suspensions in this medium was not significantly different ($P>0\cdot05$) from that of cells in the standard medium. Group 3 cells showed wide variations in size in the HPC medium. The oxygen uptake of bone marrow cells in this medium was elevated above that of cells in standard medium, but never reached significance level any time during the 20-min incubation period.

Oxygen consumption by the bone marrow cells was maximal with serum as the suspending medium (Fig. 3). Serum osmolarity as determined by a Fiske osmometer was found to be approximately 300 m-osmoles/l.

No changes in pH of the different suspending media was detected at the termination of any of the experiments.

Discussion

It is well established from previous studies that several biochemical pathways may contribute to the total oxygen uptake by blood cells (Beck 1958; Rossi, Zatti, and Zopp 1965). On this basis, variations in oxygen consumption of blood cells under different conditions of incubation could reflect both qualitative and quantitative changes in the oxidative pathway of carbon degradation. Beck (1958) indicated that respiration of polymorphonuclear leucocytes incubated in phosphate medium is mainly related to operation of the phosphogluconate oxidation pathway. Rossi, Zatti, and Zopp (1965) demonstrated increased activity of the hexose monophosphate pathway for glucose oxidation of polymorphonuclear leucocytes in the presence of serum or bicarbonate.

Results obtained with NC and NP media are not surprising when it is considered that the bicarbonate ion is the main buffer found in the blood. Apparently this ion has been integrated into the cellular oxidative mechanism to the extent where it serves as a metabolite. Lack of bicarbonate ion and increase in osmolarity of the standard medium both produced an unlike but significant ($P<0\cdot01$) reduction in oxygen uptake. Fluctuations in cell size under these two conditions were also dissimilar, with the greater fluctuation being associated with increased osmolarity of the medium. The variations in cell size with increased osmolarity could well be the result of an upset in the cellular Na^+ : K^+ balance.

The depression in oxygen uptake in media lacking phosphate is not unexpected in view of the well-known role of this ion in oxidative metabolism (Chanutin and

Curnish 1967). The increase in size of group 3 cells as a consequence of lowered osmolarity and absence of phosphate ion is of interest. The extent to which oxygen uptake is depressed is very similar in both cases. Lack of inorganic phosphate would ultimately lead to a reduction in ATP synthesis resulting in energy loss for maintenance of cell structure and shape. Likewise the swelling induced by lowering the osmolarity would be expected to disrupt cellular organization. Both conditions would produce essentially the same end result, namely a disruption of the structural detail of the cell and its ability to perform essential metabolic processes.

Cells incubated in standard, HPS, and BC media have a similar oxygen uptake. While cell volumes are somewhat increased in HPS and BC media they are not significantly different from those in Tyrode's medium. Apparently the reduced amounts of bicarbonate and phosphate ions in the BC medium do not appreciably affect oxygen consumption and cellular volume. Comparison of results obtained with NC, NP, and BC media would indicate that bicarbonate and phosphate ions exercise some effect on oxygen uptake of rat bone marrow cells. The data shows that bicarbonate has a greater effect on oxygen uptake, while phosphate has a greater effect on cell volume.

Comparison of cell volume changes in hypotonic OS 200 and hypertonic OS 400 media indicates that there is no direct relation between surface area and oxygen consumption for bone marrow cells. Large increases in surface area of cells in hypotonic medium are not accompanied by a correspondingly large increase in oxygen uptake, but rather are accompanied by a reduction in oxygen uptake. In the hypertonic medium a slight decrease in cell surface area was associated with a more pronounced reduction in oxygen consumption. This does not hold with the usual concept of surface to volume ratio where on a strictly physicochemical surface basis it would be expected that the oxygen consumption would be greater for the cells suspended in the OS 400 medium. This is further illustrated by making a comparison of cells suspended in the HPS, BC, and HPC media. These cells showed an oxygen consumption similar to the cells in the standard medium. The greatest fluctuation in cell volume occurred with group 3 cells in HPC medium; however, this fluctuation never exceeded 7% in either direction. It is clear that changes in cell volume and oxygen consumption occur simultaneously, although we could not establish a definite relation between the two events.

Good *et al.* (1966) have shown that when the buffer HEPES was utilized in homogenates a greater oxidative response was demonstrated. The increased response was attributed to the buffering ability of HEPES and not to any coupling–uncoupling effect. The present study indicates that intact cells do not show an enhanced oxidative response in the presence of HEPES alone. These cells have a consumption not significantly different from cells in Tyrode's medium. On the basis of measurements of oxygen consumption and cell volume HEPES is a good buffer.

An apparent synergistic effect on oxygen consumption is obtained when the buffer HEPES is used in conjunction with the usual bicarbonate and phosphate buffers. This results in an increased but not quite significant oxygen utilization by bone marrow cells. It is recognized that one cannot drastically reduce the amount of buffering ions in a buffered salt solution without sacrificing the buffering capacity. HEPES maintains a stable buffer system without significantly interfering with oxygen

consumption and cell size (HPS medium), and consequently permits manipulations of ions that perhaps serve both as buffers and metabolites. One wonders if the increased oxygen consumption in HPC medium might not be due to the fact that HEPES is the major buffer component of the medium, thus freeing bicarbonate and phosphate ions from buffering activity and making them more available as metabolites.

The heterogeneity of the bone marrow cell population in terms of cell types complicates interpretation of the data. Measurements of oxygen uptake were made on the entire bone marrow population. In contrast, measurements of cell volume were based on three groups of cells arbitrarily selected from the total population strictly on a size basis. In almost all instances cell volume changes were associated with group 3 cells. Since it was not possible to determine the relative contribution of this group of cells to the total oxygen consumption of the population, one cannot make any generalizations regarding correlations between changes in oxygen consumption and cell volume.

The polarographic method is well suited to making oxygen consumption determinations in a short period of time with small samples. The rapidity with which oxygen consumption can be determined by the polarographic method allows one to obtain data not possible by the use of conventional manometeric methods. There is no need for an extended temperature equilibration time and mechanical manipulations are few with the polarographic method. Consequently initial responses and adjustments of cells to alterations in the suspending medium can be studied.

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