Journal of Thermal Analysis and Calorimetry, Vol. 79 (2005) 737–740

THE USE OF ISOPERIBOL CALORIMETRY FOR THE STUDY OF AGE-RELATED CHANGES IN GLUCOSE UPTAKE BY ERYTHROCYTES

D. E. Wurster[*1, 2] *and R. Bandopadhyay*[1]

[1]Division of Pharmaceutics, College of Pharmacy, University of Iowa, Iowa City, IA 52242, USA
[2]Obermann Center for Advanced Studies, Oakdale Hall, University of Iowa, Iowa City, IA 52242, USA

Isoperibol solution calorimetry was used to determine *in vitro* erythrocyte glucose uptake changes as a function of erythrocyte age. Glucose concentrations were determined by phosphorylation with Mg^{2+}/ATP^{2-} in the presence of hexokinase. The differential heat of the standard reaction was found to be –70.3 kJ mol^{-1} (–16.8 kcal mol^{-1}). Lower amounts of glucose were taken up by 'expired,' older than 90 days, cells (0.37 mmol/5 mL packed cells) than by 'fresh,' immediately after blood collection, cells (0.87 mmol/5 mL packed cells). Statistical analyses of the data revealed significant differences in extent of glucose uptake between fresh cells and expired cells ($p<0.05$).

Keywords: *erythrocytes, glucose transport, isoperibol calorimetry*

Introduction

Isoperibol solution calorimetry has been used for the determination of enthalpies of reactions, heat capacities, heats of solution, heats of wetting and quantification of crystal polymorphism. A survey of the calorimetric literature has revealed that, in spite of its widespread use, there has been little use of this technique to follow biological processes that may change with cell aging. In this work, isoperibol calorimetry was used to study certain age-related changes associated with the red blood cell surface.

The human red blood cell (RBC) possesses a specialized transport system for sugars. Glucose transporters, which are responsible for this glucose transport, are a family of integral glycoproteins found on the RBC surface [1]. Glucose uptake by RBCs is amongst the various processes influenced by aging [2]. Normal aging is associated with impairment of glucose homeostasis [2]. Traditional methods for studying glucose transport utilize colorimetric methods. Spectroscopic methods are limited by the concentration range that can be used for analysis, and also by the presence of color-producing impurities and insoluble particulates. Glucose concentrations have been determined by phosphorylation in the presence of hexokinase using Mg^{2+}/ATP^{2-} as the phosphorylating agent (3). The heats of phosphorylation were determined by isoperibol calorimetry. The purpose of this paper is to extend the applicability of isoperibol calorimetry to the study of age-related changes in glucose uptake by erythrocytes.

Experimental

A Tronac Model 450 Isoperibol Solution Calorimeter was used to measure the heats of reaction. Prior to each run, the thermistor bridge voltage was zeroed at the temperature of the bath. The bridge imbalance signal resulting from the reaction was amplified, digitized, and recorded by a dedicated computer. Initial and final system heat capacities (effective) were determined by electrical heating.

'Fresh' (cells obtained immediately after blood collection) and 'expired' (cells older than 90 days after blood collection) human erythrocytes were obtained and suspended in tris-buffered saline. For the fresh cells, appropriate centrifugation procedures were employed to ensure that packed erythrocytes were obtained. Expired cells and fresh cells were incubated with various concentrations of glucose. After an incubation period of 1.5 hours, the cell suspensions were centrifuged and the supernatant solutions were analyzed for their glucose contents. Phosphorylating agent was prepared using the modifications to the method described by McGlothilin and Jordan [3]. Calorimetric results were compared to results obtained by a traditional spectroscopic method [4].

Statistical analyses

Analysis of variance (ANOVA) followed by multiple comparison tests (Tukey and Scheffe) was performed in order to test whether the difference in glucose uptake between the fresh and expired cells was significant.

* Author for correspondence: dale-e-wurster@uiowa.edu

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

Dummy variable analysis chart

For the purpose of the analysis, the dummy variables were defined in the following manner.

Age	Z1	Z2
Standard	0	0
Fresh cells	0	1
Expired cells	1	0

The full model can be defined as:

Heat of reaction = b0 + b1*Amount + b2*Z1 + b3* Z2 + b4*Amount*Z1 + b5*Amount*Z2

Applying the above cell coding for the dummy variables, the following equations are obtained for the three samples:

Standard model:

Heat of reaction = b0 + b1 * Amount

Fresh cells model:

Heat of reaction = (b0+b3) + (b1+b5) * Amount

Expired cells model:

Heat of reaction = (b0 + b2) + (b1 + b4) *Amount

where, 'heat of reaction' refers to the integral heats of reaction obtained by calorimetry and 'amount' is the amount of glucose that is used for the reaction.

Testing of the full model was done using the SAS program (Cary, NC).

Results

The standard curve used for the determination of glucose concentrations is shown in Fig. 1. The differential heat of reaction for the standard curve was found to be –70.3 kJ mol^{-1} (–16.8 kcal mol^{-1}), which is in close agreement with the reported value (–16 kcal mol^{-1}) for this reaction [5]. The standard curve was then used for estimating the amount of glucose in the various samples after incubation with RBCs. Heats of reaction were obtained by reaction of the supernatant acquired after incubation of a constant volume of packed cells with different concentrations of glucose.

The regression line obtained after incubation with 5 mL of expired cells (Fig. 2a) has a slope of –0.0913 cal mg^{-1}, which is close to that obtained with the standard curve (–0.0933 cal mg^{-1}). For expired cells, the displacement of the line from that of the standard curve, as is evident by the y-intercept value, indicates that there is only a small amount of glucose

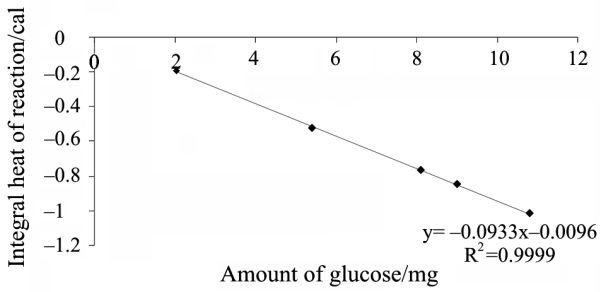

Fig. 1 Standard curve for glucose phosphorylation, as measured by calorimetry

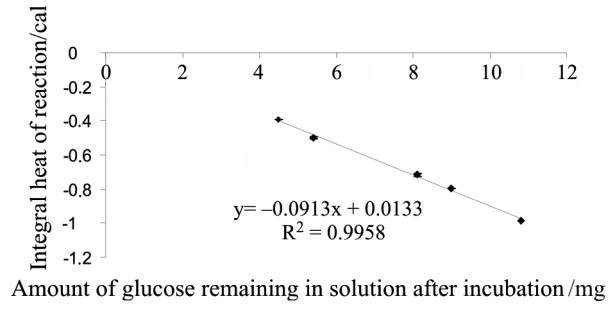

Fig. 2a Plot of integral heat of reaction *vs*. amount of glucose remaining after incubation with expired cells (5 mL)

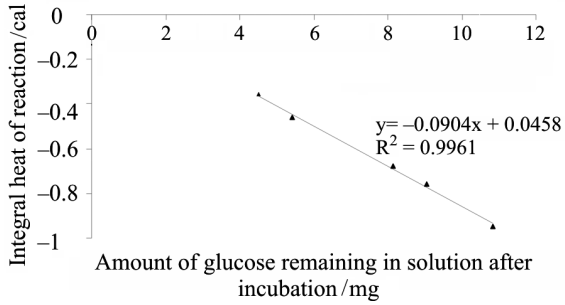

Fig. 2b Plot of integral heat of reaction *vs*. amount of glucose remaining after incubation with fresh cells (5 mL)

uptake. This is expected, since expired RBCs have been shown to have a lower glucose uptake rate than fresh cells [6]. The differential heat obtained from the plot of integral heat *vs.* concentration of glucose is –68.6 kJ mol^{-1} (–16.4 kcal mol^{-1}).

Figure 2b shows the calorimetric study of supernatant samples obtained by incubation of constant volumes (5 mL) of fresh erythrocytes with glucose solutions of varying concentrations. The regression line for the fresh cells had a slope of –0.0904 cal mg^{-1}, yielding a differential heat of –68.2 kJ mol^{-1} (–16.3 kcal mol^{-1}). The standards and the samples are both analyzed using the phosphorylation reaction. Therefore, it is expected that the differential heats obtained from the standard plot and the sample plots will be the same. This is indeed the case, and indicates that the

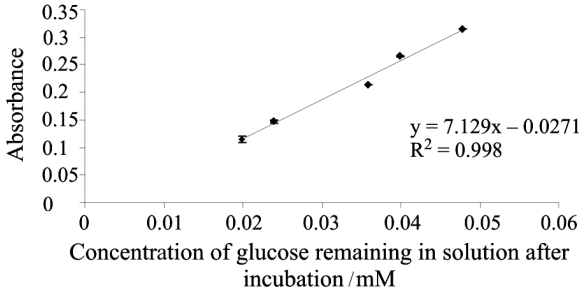

Fig. 3a Plot of absorbance *vs*. concentration of glucose remaining after incubation with expired cells

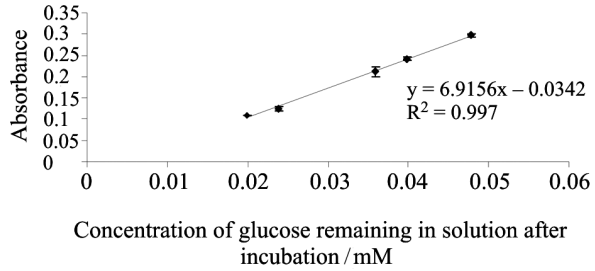

Fig. 3b Plot of absorbance *vs*. concentration of glucose remaining after incubation with fresh cells

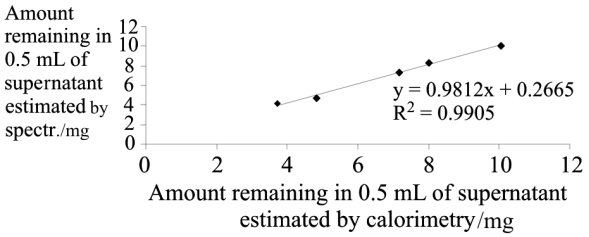

Fig. 4a Correlation between amounts remaining in the supernatant solutions after incubation with fresh cells (estimated by calorimetry and spectroscopy)

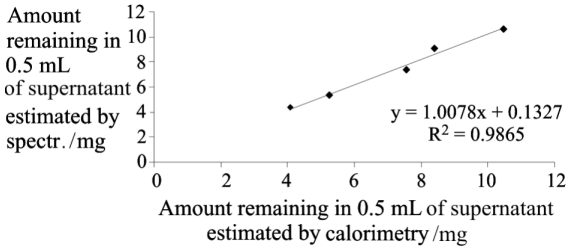

Fig. 4b Correlation between amounts remaining in the supernatant solutions after incubation with expired cells (estimated by calorimetry and spectroscopy)

analysis was performing consistently across all sample types. The plots have differing y-intercepts, depending upon the extent of glucose uptake. It can be seen that the fresh erythrocytes decrease the glucose remaining in the supernatant substantially.

The glucose oxidase method for the determination of glucose concentration was chosen as the standard method for comparison of the results obtained by calorimetry. All of the absorbance values were obtained at a wavelength of 445 nm, which was determined to the λ_{max} value. The plots of absorbance *vs*. concentration of glucose (Figs 3a and 3b) show the same trend as was seen for the calorimetry results.

The statistical results (refer to Table 2) indicate that b0 and b2 are insignificant, whereas b3 is significant. Thus, it may be concluded that the y-intercept obtained using the fresh cells in the incubation medium is significantly different from that obtained by using expired cells in the medium. Also, it is evident that b4 and b5 are insignificant. Therefore, it may be concluded that, as expected, the slopes obtained from the three samples are not statistically different from one another.

Table 1 Summary table of results obtained from calorimetry

	Slope/cal mg^{-1}	*Y*-Intercept/cal	Differential heat of reaction/ kJ mol^{-1}, (kcal mol^{-1})
Standard curve	–0.0933	–0.0096	–70.3, (–16.8)
Incubation with expired cells	–0.0913	0.0133	–68.6, (–16.4)
Incubation with fresh cells	–0.0904	0.0458	–68.2, (–16.3)

Table 2 Table of statistics for the dummy variable analysis for testing the statistical equality of slopes and y-intercepts

Variable	Parameter estimate	Standard error	*T* value	*Pr* > |*t*|
Intercept	–0.01242	0.01674	–0.74	0.4666
Amount	–0.09294	0.00218	–42.71	<0.0001
Z1	0.01730	0.02501	0.69	0.4971
Z2	0.05238	0.02502	2.09	0.0492
XZ1	0.00299	0.00329	0.91	0.3740
XZ2	0.00388	0.00329	1.18	0.2514

Table 3 Table of statistics for analysis using Tukey's studentized range for comparison between *y*-intercepts (significant differences indicated by ***) Alpha: 0.05; minimum significant difference: 0.0168 (Group 1: Satandard curve, Group 2: Expired cells, Group 3: Fresh cells)

Comparison between groups	Difference between means	
3–1	0.058400	***
3–2	0.060200	***
1–3	–0.058400	***
1–2	0.001800	
2–3	–0.060200	***
2–1	–0.001800	

Table 4 Table of statistics for analysis using Scheffe's Test for comparison between y-intercepts (significant differences indicated by ***), Alpha: 0.05; Minimum significant difference: 0.0176; (Group 1: Standard curve, Group 2: Expired cells, Group 3: Fresh cells)

Comparison between groups	Difference between means	
3–1	0.058400	***
3–2	0.060200	***
1–3	–0.058400	***
1–2	0.001800	
2–3	–0.060200	***
2–1	–0.001800	

The results obtained after testing for differences between the y-intercepts (Tables 3 and 4) indicate that the y-intercept obtained using the fresh cells is significantly different from the y-intercepts obtained with the expired cells or with the standard solutions. There was no significant difference between the latter two. Both the Tukey and Scheffe methods yield the same conclusion.

Discussion

Some investigators have shown that RBCs exhibit a progressive decrease in glucose uptake from Day 21 (after blood collection) onward, and a marked decrease at Day 42 (after blood collection, 6). It was, therefore, expected that the total amount of glucose taken up by the expired cells would be significantly lower than the total amount taken up by the fresh cells. The expired cells that were used for this study were well beyond the 42-day limit, which is the generally-accepted expiration time for human RBCs. The calorimetric data indicate that there is some small amount of glucose uptake by the expired erythrocytes. This amount was calculated to be 0.37 millimol/5mL of packed cells when incubated with a 120 mM glucose solution. Fresh cells, on the other hand, show a greater rate of glucose uptake. The amount of glucose taken up by the fresh cells was calculated to be about 0.87 mmole/5mL of packed cells. This translated into a 135% increase in glucose transport compared to the expired cells.

The parallel UV analyses of the fresh cells indicated that 0.88 millimole of glucose was taken up per 5 mL of cells. This result is in excellent agreement with the calorimetry results. Figures 4a and 4b (fresh cells and expired cells, respectively) illustrate that the calorimetric and spectroscopic techniques are in good agreement with each other, since the correlation plots have slopes very close to 1 and y-intercepts that are small. The foregoing generates further interest in the development of calorimetry as an analytical tool for studying glucose transport.

References

1 N. Kozlovsky, A. Rudich, R. Potashnik and N. Bashan, Free Radical Biology and Medicine, 23/6 (1997) 859.
2 M. Guven *et al.* Gerontology, 45 (1999) 79.
3 D. C. McGlothilin and J. Jordan, Clinical Chemistry, 21/6 (1975) 741.
4 E. Raabo and T. C. Terkildsen, Scandinav. J. Clin. and Lab. Investigation, 12 (1960) 402.
5 M. Lucia Bianconi, J. Biol. Chem., 278/21 (2003) 18709.
6 A. Janoshazi and A. K. Soloman, J. Membrane Biol., 132 (1993) 167.

Received: October 5, 2004
In revised form: December 20, 2004