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A microcalorimetric study of human erythrocytes in stirred buffer suspensions

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Abstract

The basal heat production rate P of human erythrocytes incubated in stirred buffer suspensions at pH 7.4 and 37.00 ± 0.01°C was found to be 95.8 ± 2.5 mW l^{-1} (of packed erythrocytes). A lower P value of 86.5 ± 1.9 mW l^{-1} was observed under static conditions. Within experimental errors, the measured P values could be accounted for by the heat production of chemical reactions related to the catabolic metabolism of exogenous glucose via the hexose monophosphate shunt and the glycolytic pathway. The increase in P value with pH was found to be 139 ± 4 mW l^{-1} per pH unit in the pH range 7.1–7.5, corresponding to a relative variation of 139% per pH unit. A decrease in heat production rate with cell concentration was also observed (-0.56 ± 0.14 mW l^{-1} per vol.% of erythrocytes).

INTRODUCTION

Mature erythrocytes are relatively simple, non-growing cells without nuclei or mitochondria. Because they lack respiratory capacity, their maintenance energy and reducing equivalents are derived from glucose via the hexose monophosphate shunt (HMPS) and the glycolytic pathway [1]. Human erythrocytes were among the first mammalian cells to be studied by microcalorimetry. The pioneering study of Levin and Boyo in 1971 [2] has been followed by a large number of microcalorimetric investigations on erythrocytes; some of these studies have focused on the potential clinical applications of the method in diagnosing such diseases as anemia and hyperthyroidism [2–6] and others have concentrated on methodological issues [3,7–9]. Because the molecular basis of erythrocyte heat production still remains largely unknown, the findings of most calorimetric studies have been interpreted on a phenomenological level using the calorimetrically determined heat production rate ($P = dQ/dt$) as a general measure

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of metabolic activity. Several workers have attempted to relate calorimetric data to results from conventional metabolic investigations [6,10–13], although none has been able to account quantitatively for the measured heat production in terms of metabolic reactions.

The purpose of the present investigation was to obtain better knowledge of the main heat-producing processes in human erythrocytes, thus facilitating more detailed interpretations of calorimetric results. Calorimetry results have therefore been related to the metabolism of exogenous glucose using enthalpy values calculated from literature data. During measurements, erythrocytes were incubated in stirred homogeneous buffer suspension. The effect of such experimental variables as pH, cell concentration and stirring has also been determined.

EXPERIMENTAL

Preparation of erythrocytes

Venous blood was collected from healthy male donors in 10 ml heparinized tubes (Vacutainer™, Becton–Dickinson, France). Leukocytes and platelets were removed by passing the blood through a column containing a 1 : 1 mixture of microcrystalline cellulose (Sigmacell) and α-cellulose (Sigma Chemical Company, St. Louis, MO, USA), essentially as described by Beutler et al. [14]. The erythrocytes were eluted with approximately two volumes of cholin-Ringer buffer (cholin chloride 146 mM, $MgCl_2$ 1 mM, $CaCl_2$ 1 mM, NaCl 5 mM, orthophosphoric acid 2.5 mM, pH 7.4), washed by repeated centrifugations (5 min at 1000 g) and resuspended in Hank's balanced salt solution (Hank's BSS) containing 5.5 mmol l^{-1} glucose. The suspensions were buffered with 20 mmol l^{-1} HEPES (sodium bicarbonate was excluded from the media). Erythrocyte preparations were made at ambient temperature (22–24°C) and measurements were started within 2 h of the blood sample being collected.

Calorimetry

The heat production rate was measured using a 4-channel heat conduction calorimeter (Thermal Activity Monitor, Thermometric AB, Järfälla, Sweden), equipped with titration/perfusion vessels [15,16]. In each vessel, samples of 2.7 ml of erythrocytes were kept in homogeneous suspension by means of a 'turbine' stirrer [17] operating at a rate of 90 rev min^{-1}. A gas phase of 0.7 ml (air) was present above the cell suspension. All calorimetric measurements were made at 37.00 ± 0.01°C. Initial values of pH and cell concentration were adjusted to pH 7.4 and a hematocrit value (vol.% of erythrocytes) of 20%, respectively, if not otherwise stated. The total heat production rate was determined together with pH and cell concentration

values after an incubation period of 1.5 h, at a point when calorimetric measurements were terminated. The pH was measured with a Radiometer G297/62 capillary electrode (Radiometer, Copenhagen, Denmark) and cell concentrations were determined in duplicate using a microhematocrit centrifuge (Hettich, Germany). Instrument baseline values were obtained between each measurement with 2.7 ml water in the vessel. The instrument was calibrated by means of an insertion heater [18].

The effect of medium pH on the heat production rate of erythrocytes was determined in the pH range 7.1–7.5. Experiments consisted of four parallel measurements with a different pH value in each of the four erythrocyte suspensions. The effect of variation in the cell concentration was determined in an analogous manner for a hematocrit range of 5%–45%.

Results obtained with the stirred vessel were also compared with results obtained in parallel experiments using a 3 ml static vessel [15] of a type frequently used in earlier measurements [4,5,7,8,19,20]. In measurements with the static vessel, the pH was determined in the erythrocyte sediment layer.

Metabolic analyses

The contribution of glucose metabolism to the measured heat production rate was assessed in a series of experiments where changes in the levels of CO_2, glucose, lactate and pyruvate were determined in experiments paralleling the calorimetric measurements. Samples for metabolite analysis were taken at regular intervals during 3 h from erythrocyte suspensions incubated at 37.0 ± 0.1°C in a shaking water bath.

The oxidation of glucose was followed by the production of $^{14}CO_2$ from D-(u $-$ ^{14}C) glucose (Amersham International, Amersham, UK). Erythrocytes were incubated in the presence of 30 MBq l^{-1} D-(u $-$ ^{14}C) glucose in sealed 50-ml Erlenmeyer flasks, each containing 5 ml of cell suspension. The carbon dioxide was adsorbed in a plastic cup containing a filter paper soaked in 250 ml of Hyamine hydroxide (Packard, Downers Grove, IL, USA). The cup was attached to a gas-tight rubber stopper. The incubation was terminated at regular intervals by the injection of 2 ml of 15% H_2SO_4(aq) (w/w) into one of the flasks. After an additional incubation period of 2 h, the flask was removed from the water bath and the plastic cup transferred to a scintillation vial and analyzed for carbon-14 activity using a 1211 Rackbeta Scintillation counter (LKB Wallac, Bromma, Sweden).

Samples for analysis of condensed metabolites were incubated in disposable cell culture tubes. The incubation was stopped at regular intervals by transferring the test tube from the water bath to a container of dry ice. The frozen samples were stored at -20°C overnight, thawed in an ultrasonicator and deproteinized by ultrafiltration (Centrifree™, Amicon Corp., USA).

The filtrates were analyzed for glucose, lactate and pyruvate using an isocratic HPLC-system equipped with refractometer detector (LKB 2142, Bromma, Sweden). Metabolites were separated at 45°C on an Aminex HPX-87H column (BioRad, Richmond, CA, USA) using 6.5 mmol l^{-1} H_2SO_4(aq) (flow rate = 0.6 ml min^{-1}) as mobile phase.

Data processing and presentation

Results from different experiments shown in Figs. 1 and 2 were normalized, the factor used being the quotient of the y estimate (at $\bar{x}$) calculated for the single experiment, divided by the y estimate (at $\bar{x}$) calculated for all values; y estimates were obtained by linear regression analysis based on a least-squares method. All errors are given as $\pm$ the standard error of the mean (S.E.M. $= s/\sqrt{n}$). Heat production rates P are presented in units of mW per liter of packed erythrocytes (mW l^{-1}). Metabolic changes are presented in units of 10^{-6} mol per second and liter of packed erythrocytes (μmol s^{-1} l^{-1}). Cell concentration (hematocrit) values are presented as vol.% of packed erythrocytes.

RESULTS

Variation in heat production rate with pH and cell concentration

In Fig. 1, normalized P values (in mW l^{-1} (of packed erythrocytes) from six consecutive experiments are plotted against the medium pH. P values were obtained using the stirred vessels. The average slope was 139 ± 4 mW l^{-1} per pH unit, as calculated by means of linear regression analysis. The following equation was derived to correct P values for variations in pH

$$P' = P + 139(7.4 - \mathrm{pH}) \quad (1)$$

where P' is the P value as the 'standard' pH of 7.4. Using the evaluation

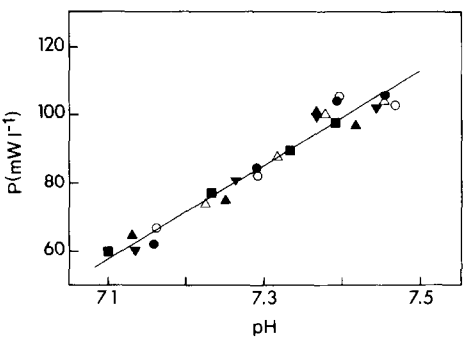

Fig. 1. Variation in heat production rate with medium pH. P values were normalized as described in the text.

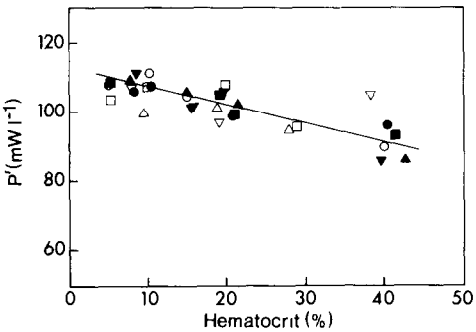

Fig. 2. Variation in heat production rate with cell concentration (in % (v/v)). P values were normalized and corrected to pH 7.4 as described in the text.

method adopted by Monti and Wadsö (based on a plot of the relative heat production rate ($P_r = 100P/P'$) versus pH) [7], the P value was found to vary by 139% per pH unit. The average hematocrit value was $20.0 \pm 0.2\%$.

Figure 2 shows a plot of normalized P' values (corrected to pH 7.4 using eqn. (1)) versus cell concentration. Values were obtained with stirred vessels in 8 consecutive experiments. The P' values varied by an average of -0.56 ± 0.14 mW l^{-1} per hematocrit unit, as calculated by linear regression analysis. The following equation was derived to correct P values for variations in cell concentration

$$P'' = P - 0.56(20 - C) \quad (2)$$

where P'' is the P at the 'standard' hematocrit value of 20% and C is the experimental hematocrit value.

Equations (1) and (2) were combined (eqn. (3)) and used to correct all the subsequent P values for variations in pH and cell concentration

$$P^{\ominus} = P + 139(7.40 - \text{pH}) - 0.56(20 - C) \quad (3)$$

where $P^{\ominus}$ denotes the P at the 'standard' values (pH 7.4 and hematocrit 20%). The effects of pH and cell concentration on the measured P value were assumed to be independent of each other.

Differences between values obtained with static and stirred methods

Table 1 shows results from six experiments where $P^{\ominus}$ values obtained with static and stirred vessels are compared, from which it can be seen that significantly higher $P^{\ominus}$ values were obtained with the stirred vessel. Typical calorimetric records from experiments with stirred and static vessels are shown in Figs. 3A and 3B, respectively. Stirred and static vessels were inserted in the calorimeter (as indicated by the arrows), reaching measuring position after equilibration periods of 40 and 20 min, respectively. A slow decrease in heat production rate with time ($4.1 \pm 1.4\%$ h^{-1})

TABLE 1

Comparison of heat production rates obtained with static vs. stirred vessels [a]

	Hematocrit (%)	Static vessel		Stirred vessel		Difference [b] (%)
		pH	$P^{\ominus}$ (mW l^{-1})	pH	$P^{\ominus}$ (mW l^{-1})	
	19.7	7.356	81.0	7.358	86.4	6.6
	19.0	7.416	84.7	7.416	91.8	8.3
	18.0	7.380	83.6	7.380	95.7	14.5
	18.3	7.347	86.9	7.339	100.5	15.6
	17.8	7.356	89.3	7.353	97.9	9.7
	18.3	7.385	93.7	7.379	102.6	9.5
Mean	18.5	7.373	86.5 ± 1.9	7.371	95.8 ± 2.5	10.7 ± 1.5

[a] All $P^{\ominus}$ values are corrected as described in the text. Errors are given as ± S.E.M.
[b] The difference is calculated as 100 $((P^{\ominus}_{stir}/P^{\ominus}_{stat})-1)$.

was observed in all cases, irrespective of the measuring method used. During measurements, the medium pH decreased with an average value of 0.042 ± 0.005 units per h as a result of cellular metabolism. Using eqn. (1), the observed value for pH reduction can be estimated to result in a reduction in heat production rate of 6.2 ± 0.8% h^{-1} (errors are given at a confidence level of 0.95).

Changes in metabolite levels

Results from metabolite analyses are shown in Table 2 (in μmol s^{-1} l^{-1}). Within experimental errors, all of the consumed glucose carbon could

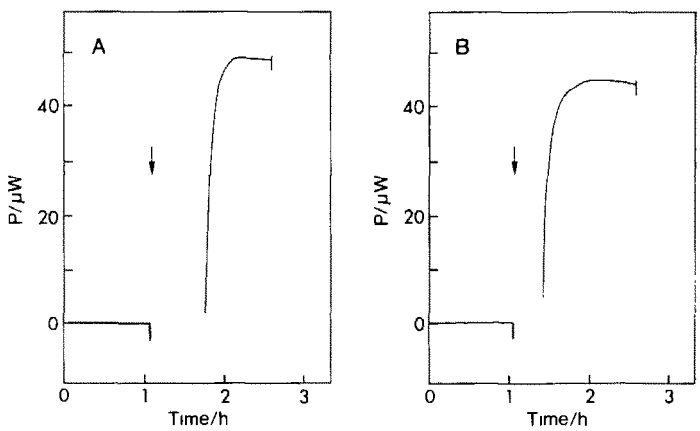

Fig. 3. Calorimetric records of heat production rate vs. time obtained with a stirred vessel (curve A) and with a static vessel (curve B), each containing 2.7 ml of a 20% (v/v) erythrocyte suspension at pH 7.4. Stirred and static vessels were inserted in the calorimeter as indicated by the arrows, reaching measuring position after equilibration periods of 40 and 20 min, respectively.

TABLE 2

Results of metabolite analysis of erythrocytes incubated in suspension

	Metabolite changes	
	(nmol l^{-1} s^{-1}) [a]	(%) [b]
Glucose	−404 ± 71 (5)	–
CO_2	+24 ± 4 (6)	1.0 ± 0.2
Lactate	+804 ± 48 (6)	99 ± 12
Pyruvate	+41 ± 7 (5)	5.1 ± 1.1

[a] Accumulation is indicated by a (+) sign, consumption by a (−) sign. Errors are given as ± S.E.M. Numbers within brackets indicate number of determinations.

[b] Values indicate the percentage of glucose carbon retrieved in the compound.

be retrieved in CO_2 (1.0 ± 0.2%), lactate (99 ± 12%) and pyruvate (5.1 ± 1.1%). No significant change in the concentration of inorganic phosphate was observed (not shown).

Thermochemical calculations

The heat production rate P_{cal} of stirred erythrocyte suspensions was found to be 92.6 ± 2.7 mW l^{-1} at a pH value of 7.37 ± 0.02 and a hematocrit value of 18.5 ± 0.3% (Table 4). Using eqn. (3), $P_{cal}^{\ominus}$ was calculated as 95.8 ± 2.5 mW l^{-1}. The theoretical P values for metabolism of

TABLE 3

Metabolic reactions and the corresponding enthalpy changes [a]

Reaction	$\Delta_r H_m$ (kJ mol^{-1})
(a) $C_6H_{12}O_6(aq) + 6O_2(g) + 6B(aq) \rightarrow 6HCO_3^-(aq) + 6B\text{–}H^+(aq)$	−3044
(b) $C_6H_{12}O_6(aq) + 2B(aq) \rightarrow 2CH_3CH(OH)COO^-(aq) + 2B\text{–}H^+(aq)$	−161
(c) $C_6H_{12}O_6(aq) + O_2(g) + 2B(aq) \rightarrow 2CH_3COCOO^-(aq) + 2H_2O(aq) + 2B\text{–}H^+(aq)$	−552

[a] The $\Delta_r H_m$ values in this table refer to 298.15 K and the molecular species stated. The experimental processes are not quite identical to those used to obtain the values in the table with respect to temperature (measurements were performed at 310.15 K) and molecular species (acid–base equilibria), but the combined effects of these differences on the calculated $\Delta_r H_m$ values are not significant in the present context. Enthalpy values used in the calculations are obtained from Wilhoit (ref. 21). The protonation enthalpy is assumed to be −26 kJ mol^{-1}, because the distribution of H^+ between the extra-cellular buffer HEPES ($\Delta_r H_m = -21.7$ kJ mol^{-1} [22] and the intra-cellular buffer (presumably histidine groups [23] with a $\Delta_r H_m$ of −30 kJ mol^{-1} [24,25] is unknown.

TABLE 4

Measured and calculated heat production rates [a]

P_{cal}	$P^{\ominus}_{cal}$	P_a	P_b	P_c	$\Sigma(P_{(a)-(c)})$	$\Sigma(P^{\ominus}_{(a)-(c)})$
92.6	95.8	12.2	64.9	11.3	89.1	92.2
±2.7	±2.5	±2.0	±3.9	±2.0	±5.6	±5.3

[a] Values are calculated as described in the text. Errors are given as ±S.E.M.

exogenous D-glucose via reactions (a)–(c) (Table 3) were calculated according to

$$P_i = v_i \, \Delta_r H_{m,i} \tag{4}$$

where v_i is the reaction rate obtained from the rate of end-product accumulation (Table 2) and $\Delta_r H_{m,i}$ is the corresponding enthalpy change (i = (a), (b) or (c)). The reactions postulated in Table 3 are based on the assumption that O_2 is the ultimate electron acceptor in all redox processes (see discussion). Results show that the heat production rates calculated from the breakdown of glucose to CO_2, lactate and pyruvate ($P_{(a)-(c)}$ in Table 4), account for 13%, 70% and 12%, respectively, of P_{cal}. Within experimental errors, the sum of the calculated P values $\Sigma(P_{(a)-(c)})$ is identical to P_{cal}. The 'standard' value of $\Sigma(P_{(a)-(c)})$ ($\Sigma(P^{\ominus}_{(a)-(c)})$) was calculated using eqn. (3).

DISCUSSION

The observed relative variation in heat production rate with medium pH (139% per pH unit) is in fair agreement with the value observed by Monti and Wadsö (120% per pH unit) for erythrocytes suspended in autologous plasma under static conditions [7]. Because the HMPS is barely affected by pH in this range [1], most of the pH dependency can thus be related to the glycolytic pathway, especially the phosphofructokinase reaction [26]. In the present experiments, 82% of the P value could be related to glycolytic processes (Table 4), and the pH dependency of the glycolytic pathway can thus be calculated as 139%/0.82 = 169% per pH unit. This value is close to the relative variation in erythrocyte glucose consumption rate observed by Murphy (160%) [1] in the pH range 7.1–7.8. The division of erythrocyte heat production into a pH-dependent and a non-pH-dependent part makes comparison between pH effects on the P value measured under different conditions difficult. For example, the relative variation in P with pH is only 40% per pH unit when erythrocytes are incubated in the presence of methylene blue which activates the HMPS [19]. The observed decrease in medium pH due to cellular metabolism explains the reduction in heat production rate during the calorimetric measurements (Fig. 3).

The variation in P' values with cell concentration is relatively small (0.56% per hematocrit unit) and the possibility that it is an artifact can not be excluded. The P' values shown in Fig. 2 are corrected for variations in medium pH but not for variations in other experimental variables, e.g. metabolite concentrations, which might be expected to change with the metabolic activity of the system. Oxygen limitations at higher cell concentrations have, for example, resulted in similar 'crowding' effects observed for human lymphocytes [27]. The presently observed 'crowding' effect might also explain the difference in $P^{\ominus}$ value between static and stirred vessels (Table 1). The observed reduction in the $P^{\ominus}$ value for the static vessel corresponds to a hematocrit difference of 17%, a difference which might very well be explained by sedimentation in the static vessel.

Results from metabolite analyses indicated that all of the glucose carbon could be retrieved in CO_2, lactate and pyruvate (Table 2), a finding consistent with the proposed pathways for glucose metabolism in these cells [1]. The accumulation of CO_2 suggests that approximately 6% of the glucose carbon passes through the HMPS, which is in fair agreement with an earlier observed value of 10% [1]. The production of pyruvate indicates a demand for NADH formed in the phosphoglyceraldehyde dehydrogenase reaction, probably for the purpose of methemoglobin reduction [28].

Basal heat production rates in the range of 75–108 mW l^{-1} have been observed from human erythrocytes incubated under static conditions (at pH 7.4 and a hematocrit value of 40%) [4,8,11,19,20,29]. The observed variation between basal P values obtained from different sources might be expected to be a result of differences in preparation methods, suspension media, protonation enthalpies, etc. [7,8,10]. The P values observed here (Tables 1 and 4) are thus in agreement with the published data. The calculation of theoretical heat production values in Table 4 assumes that cells are kept under 'steady-state' conditions during the measurements, i.e. under conditions where no net changes in cellular biomass occur. The ultimate electron acceptor in reactions (a) and (c) is therefore assumed to be oxygen (Table 3) rather than the NADPH or NADH formed directly in these reactions. In the same manner, ATP formation in the glycolytic reactions ((b) and (c) in Table 3) is assumed to be balanced by the demand for ATP in such processes as maintaining the electrochemical potential over the cell membrane. The calculated heat production rates of glycolytic reactions ($P_{(b)}$ and $P_{(c)}$ in Table 4) account for 82% of P_{cal}, while the contribution of the HMPS ($P_{(a)}$ in Table 4) was estimated as 13% of P_{cal}. The good agreement between the sum of the calculated P values $\Sigma(P_{(a)-(c)})$ and P_{cal} indicates that erythrocyte heat production under these conditions is a result of the catabolic metabolism of exogenous glucose via the HMPS and the glycolytic pathway. The amount of heat per mole of consumed glucose was calculated as 230 ± 13 kJ mol^{-1} from the values given in Tables 2 and 4. Because corresponding values calculated from primary

results in earlier studies are similar (205 ± 26 kJ mol^{-1} [8], 256 kJ mol^{-1} [10] and 216 kJ mol^{-1} [3]), it is likely that in these studies also the observed heat production rate may have been due to glucose metabolism.

Significant heat production rates (15–60 mW l^{-1}) have also been reported for glucose-depleted erythrocytes [10,12,13]. This heat production might be partly explained by net oxidation of the cellular constituents as the regeneration of NADPH and NADH is terminated. An estimate of the auto-oxidation rate might be obtained from the oxygen consumption in reactions (a) and (c) (Table 3), which can be calculated as 45 nmol s^{-1} l^{-1} using the data in Table 2. The molar enthalpy change of oxygen-consuming processes is relatively constant, with an average value of 450 kJ mol^{-1} of oxygen [30]. Using this value, the expected heat production rate due to net oxidation of biomass during glucose-depleted conditions can be estimated to 20 mW l^{-1}.

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REFERENCES

1 J.R. Murphy, Erythrocyte metabolism. II. Glucose metabolism and pathways, J. Lab. Clin. Med., 55 (1960) 286–302.
2 K. Levin and A.E. Boyo, Heat production from erythrocytes, Scand. J. Clin. Lab. Invest. Suppl., 118 (1971) 55.
3 K. Levin, Determination of heat production from erythrocytes in normal man and in anemic patients with flow microcalorimetry, Scand. J. Clin. Lab. Invest., 32 (1973) 55–65.
4 M. Monti and I. Wadsö, Microcalorimetric measurements of heat production in human erythrocytes. I. Normal subjects and anemic patients, Scand. J. Clin. Lab. Invest., 32 (1973) 47–54.
5 M. Monti and I. Wadsö, Microcalorimetric measurements of heat production in human erythrocytes. II. Hyperthyroid patients before, during and after treatment, Acta Med. Scand., 200 (1976) 301–308.
6 M. Monti, P. Hedner, J. Ikomi-Kumm and S. Valdemarsson, Erythrocyte thermogenesis in hyperthyroid patients: Microcalorimetric investigation of sodium/potassium pump and cell metabolism, Metabolism, 36 (1987) 155–159.
7 M. Monti and I. Wadsö, Microcalorimetric measurements of heat production in human erythrocytes. III. Influence of pH, temperature, glucose concentration and storage conditions, Scand. J. Clin. Lab. Invest., 36 (1976) 565–572.
8 M. Monti and I. Wadsö, Microcalorimetric measurements of heat production in human erythrocytes. IV. Comparison between different calorimetric techniques, suspension media and preparation methods, Scand. J. Clin. Lab. Invest., 36 (1976) 573–580.
9 J.A. Ikomi-Kumm, The effect of cellular flow and viscosity on heat production by human erythrocytes measured in vitro by flow reaction calorimetry, IRCS Med. Sci., 5 (1977) 320.

10 K. Levin, P. Fürst, R. Harris and E. Hultman, Heat production from human erythrocytes in relation to their metabolism of glucose and amino acids, Scand. J. Clin. Lab. Invest., 34 (1974) 141–148.
11 J.A. Ikomi-Kumm, The relationship between heat production rate, glycolysis and Na, K-ATPase activity in human erythrocytes, Ph. D. Thesis, University of Lund, Sweden, 1984.
12 S. Minakami and C-H. de Verdier, Calorimetric study of human erythrocyte glycolysis, Eur. J. Biochem., 64 (1976) 451–460.
13 J. Grarup, E. Hasselager and M. Hjelm, The heat production in energy depleted human erythrocytes induced by glucose, inosine and adenine, Scand. J. Clin. Lab. Invest., 37 (1977) 85–88.
14 E. Beutler, C. West and K.G. Blume, The removal of leukocytes and platelets from whole blood, J. Lab. Clin. Med., 88 (1976) 328–333.
15 J. Suurkuusk and I. Wadsö, A multichannel microcalorimetry system, Chem. Scr., 20 (1982) 155–163.
16 M. Görman-Nordmark, J. Laynez, A. Schön, J. Suurkuusk and I. Wadsö, Design and testing of a new microcalorimetric vessel for use with living cellular systems and in titration experiments, J. Biochem. Biophys. Methods, 10 (1984) 187–202.
17 A. Schön and I. Wadsö, Thermochemical characterization of T-lymphoma cells under non-growing conditions, Cytobios, 48 (1986) 195–205.
18 L.-E. Briggner and I. Wadsö, Test and calibration processes for microcalorimeters, with special reference to heat conduction instruments used with aqueous systems, J. Biochem. Biophys. Methods, 22 (1991) 101–118.
19 M. Monti and I. Wadsö, Microcalorimetric measurements of heat production in human erythrocytes. V. Heat effect during methylene blue stimulation, Scand. J. Clin. Lab. Invest., 36 (1976) 431–436.
20 U. Bandmann, M. Monti and I. Wadsö, Microcalorimetric measurements of heat production in whole blood and blood cells of normal persons, Scand. J. Clin. Lab. Invest., 35 (1975) 121–127.
21 R.C. Wilhoit, in H.D. Brown (Ed.), Biochemical Microcalorimetry, Academic Press, London, 1969, pp. 305–317.
22 L. Beres and J.M. Sturtevant, Calorimetric studies of the activation of chymotrypsinogen A, Biochemistry, 10 (1971) 2120–2126.
23 J. Wyman, An analysis of the titration data of oxyhemoglobin of the horse by a thermal method, J. Biol. Chem., 127 (1939) 1–13.
24 J.J. Christensen, R.M. Izatt, D.P. Wrathall and L.D. Hansen, Thermodynamics of protonionisation in dilute aqueous solutions, J. Chem. Soc. A, (1969), pp. 1212–1223.
25 M.N. Jones and C. Zala, Thermochemical studies of human red blood cells: enthalpy changes on dilution in buffers, Proc. R. Soc. London., 185 (1974) 73–82.
26 S. Minakami and H. Yoshikawa, Studies on erythrocyte glycolysis. III. The effects of active cation transport, pH and inorganic phosphate concentration on erythrocyte glycolysis, J. Biochem., 59 (1966) 145–150.
27 C.J. Hedeskov and V. Esmann, Respiration and glycolysis of normal human lymphocytes. Blood, 28 (2) (1966) 163–174.
28 Q.H. Gibson, The reduction of methemoglobin in red blood cells and studies on the cause of idiopatic methemoglobinemia. Biochem. J., 42 (1948) 13–18.
29 M. Monti and J. Ikomi-Kumm, Erythrocyte heat production in human obesity. Microcalorimetric investigations of sodium potassium pump and cell metabolism, Metabolism, 34 (1985) 183–187.
30 E. Gnaiger, in E. Gnaiger and H. Forstner (Eds.), Polarographic Oxygen Sensors, Aquatic and Physiological Applications, Springer, Berlin, 1983, pp. 337–345.