The Measurement of Red Cell pH from the Distribution of DMO

During the past 10 years, the weak acid DMO (dimethadione; 5, 5-dimethyloxazolidine-2, 4-dione) has been widely used to measure intracellular pH[1]. The pH of the erythrocyte, for instance, has been determined from the distribution of DMO in both in vivo[2] and in vitro[3–5] conditions. Nevertheless, it has been suggested[4–6] that the application of the method to the mature red cell in vitro does not provide a valid estimate of erythrocyte pH. When the simultaneous determination of the pH of haemolysates is carried out, different values are obtained[3–5], and there are significant differences between chloride and hydrogen ion distribution in blood when the latter is based on equilibration with DMO[2]. In addition, the potential difference predicted from the distribution of DMO[2] is inconsistent with recent measurements of the membrane potential in mammalian red cells[7,8].

In the present experiments, the validity of the DMO method (as applied to mature red cells in vitro) has been reassessed. Erythrocyte pH was estimated from the in vitro distribution of DMO-2-C^{14}, and the method was evaluated by comparing hydrogen and chloride ion distribution with recent measurements of the potential difference across the red cell membrane.

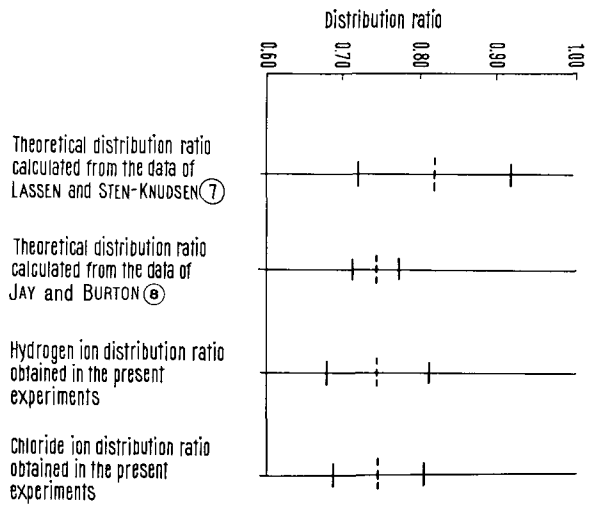
Samples of blood were obtained by venepuncture or cardiac puncture and heparinized. After removal of the plasma and white cells, erythrocytes were washed and suspended in Krebs-Ringer phosphate buffer (pH 7.40). In these conditions, carbon dioxide tension is negligible, extracellular pH is stabilized throughout the entire experiment, and both hydrogen and chloride ions are in electrochemical equilibrium across the red cell membrane. The intracellular pH of the red cells was determined from the distribution of DMO-2-C^{14} as previously described by CALVEY[9]; chloride ion concentrations in erythrocytes and extracellular buffer were measured by the method of SCHALES and SCHALES[10]. Distribution ratios for hydrogen and chloride ions were calculated from the expressions

$$\frac{a\mathrm{H}^+ \text{ buffer}}{a\mathrm{H}^+ \text{ cell water}} \text{ and } \frac{c\mathrm{Cl}^- \text{ cell water.}}{c\mathrm{Cl}^- \text{ buffer}}$$

These results were compared with the theoretical distribution ratio for diffusable ions (r), which was calculated from measurements of the membrane potential (E) recently reported by LASSEN and STEN-KNUDSEN[7] and JAY and BURTON[8]. Theoretical values were calculated from the equation $r = 1/e^{-FE/RT}$, where F, R, and T have their usual significance.

In 40 experiments in normal animals, the pH of the rabbit erythrocyte was 7.27 ± 0.05 (mean $\pm$ standard deviation). The mean distribution ratios for hydrogen and chloride ions were equal (0.74), although large variations were recorded in some individual experiments (Figure). These values coincided with the theoretical distribution ratio calculated from the membrane potentials recorded by JAY and BURTON[8]. Interpolation of the results of LASSEN and STEN-KNUDSEN[7] resulted in a rather higher distribution ratio with a greater degree of variability. In the experiments of LASSEN and STEN-KNUDSEN, the membrane potential of human red cells was frequently less than 5 mV; these values were considered to be lower than the true membrane potential, due to the electrical leak induced by puncture of the cell. Similarly, the scatter of the values was attributed to imperfections in the experimental technique rather than to biological variability. Exclusion of the lowest membrane potentials recorded by LASSEN and STEN-KNUDSEN[7] (as suggested by these authors) make their data comparable with that of JAY and BURTON[8], and consistent with the distribution ratios obtained in the present study.

The concurrence between the theoretical ratio, chloride ion distribution, and hydrogen ion distribution based on equilibration with DMO in vitro, suggests that the latter method provides an accurate assessment of erythrocyte pH. Similar conclusions were reached in experiments (Table) in which large amounts of non-labelled DMO were added to DMO-2-C^{14} and equilibrated with red cell suspensions. Concentrations of carrier DMO 1000 times greater than the labelled compound failed to influence the distribution of radioactivity; these experiments effectively eliminate any possibility of active transport or intracellular binding of DMO in red cell suspensions. In fact, the difficulties and assumptions involved are much less when DMO is used to determine erythrocyte pH, than when the method is applied to other tissues. No limitations are imposed by the possible permeability of the cell membrane to the DMO anion; considerations of structural inhomogeneity are of little importance, in spite of the Donnan equilibrium; and the cellular and extracellular phases of blood cell suspensions can be easily separated and analyzed. Indeed, it is difficult to criticize the use of DMO as an indicator of intracellular pH in erythrocytes without questioning its use in other tissues, since similar principles are involved in both instances.



Comparison between the theoretical distribution ratios for diffusable ions and the hydrogen and chloride ion ratios obtained by experiment. – – –, mean; ——, standard deviation.

[1] W. J. WADDELL and R. G. BATES, Physiol. Rev. *49*, 285 (1969).
[2] P. A. BROMBERG, J. THEODORE, E. D. ROBIN and W. N. JENSEN, J. Lab. clin. Med. *66*, 464 (1965).
[3] R. THOMASON, Scand. J. clin. Lab. Invest. *15*, 45 (1963).
[4] W. R. SANSLONE and E. MUNTWYLER, Proc. Soc. exp. Biol. Med. *116*, 582 (1964).
[5] N. J. PAYMASTER and S. ENGLESSON, Acta anaesthesiol. scand. *4*, 219 (1966).
[6] J. FUNDER and J. O. WIETH, Acta physiol. scand. *68*, 234 (1966).
[7] U. V. LASSEN and O. STEN-KNUDSEN, J. Physiol. *195*, 681 (1968).
[8] A. W. L. JAY and A. C. BURTON, Biophys. J. *9*, 115 (1969).
[9] T. N. CALVEY, Life Sci. *7*, 619 (1968).
[10] O. SCHALES and S. S. SCHALES, J. biol. Chem. *140*, 879 (1941).

The results of the present experiments suggest that the normal intracellular pH of the rabbit erythrocyte is 7.27. This value is consistent with previous estimations of red cell pH in the rat[4] and in man[3]. Both these studies were based on the equilibration of non-labelled DMO with red cells in vitro. On the other hand, measurements based on equilibration with DMO in vivo[2] or on the pH of haemolysates[3–5] suggest that erythrocyte pH is 0.10–0.20 pH units lower. These latter values are not consistent with the membrane potential of the erythrocyte[7,8]. Estimation of the pH of haemolysates is unlikely to reflect true red cell pH, since the measured potential is biassed by the liquid junction potential[3,11] and the effect of hydration on electron donor and electron acceptor groups in the intact erythrocyte[12].

The effect of non-labelled DMO on the distribution of DMO-2-C^{14}

Blood cell suspension +	DMO-2-C^{14} in cell suspension (dpm/ml) (x)	DMO-2-C^{14} in separated buffer (dpm/ml) (y)	Distribution $\frac{(x)}{(y)}$
DMO-2-C^{14} (10^6 dpm)	278,300	349,600	0.80
DMO-2-C^{14} (10^6 dpm) + 0.1 mg DMO	294,800	349,300	0.84
DMO-2-C^{14} (10^6 dpm) + 1.0 mg DMO	258,500	319,700	0.81
DMO-2-C^{14} (10^6 dpm) + 10.0 mg DMO	249,900	297,200	0.84

The amount of DMO-2-C^{14} equilibrated with the blood cell suspensions was equivalent to 1–10 µg of the compound.

Zusammenfassung. Die pH-Werte von Kaninchen-Erythrozyten wurden mit Hilfe der DMO-Verteilungsmethode untersucht und das intrazelluläre pH in Übereinstimmung mit dem Membranpotential gefunden.

T. N. CALVEY

Department of Pharmacology and General Therapeutics, University of Liverpool, Liverpool L69 3BX (England), 15 October 1969.

[11] J. T. G. OVERBEEK, Progr. Biophys. biophys. Chem. *6*, 57 (1956).
[12] T. L. DORMANDY, J. Physiol. *183*, 378 (1966).

Rate of Oxidation and Tissue Concentrations of Ethanol in Mice

We have previously shown that a s.c. injection of the median lethal dose of ethanol in mice produced a tissue concentration that reduced by about one-third the thermodynamic activity of the intercellular water 2 h later without a compensating decrease in electrolyte concentration[1]. We became interested to find out precisely the concentrations of ethanol in various organs of the mouse at a given time after the injection and any significant changes in the weight of the organs.

The coefficient of ethyl oxidation (CEO), a useful indicator of the speed and nature of ethanol oxidation in a given species, is quite variable in the literature. In man the normal value for the CEO is 90 mg/kg/h[2]. In mice KINARD[3] obtained the following values: 455 ± 173.3 mg/kg/h during the first $^1/_2$ h after the injection, 555.5 ± 73.1 mg/kg/h for the first h and 595.5 ± 68.3 mg/kg/h for the first 4 h. MARSHALL and OWENS[4] reported 1322 mg/kg/h, 1123 mg/kg/h and 691 mg/kg/h respectively for the 3 periods. Finally, NELSON et al.[5] obtained a CEO of 625 mg/kg/h for the first h and 598 mg/kg/h for the first 3 h. In this paper we have determined the CEO in a group of mice of the same stock.

Ethanol at a dose of 10 g/kg was injected as a 25% solution with NaCl at 0.9% into 30 Swiss male albino mice. The animals were on a commercial diet ad libitum and had free access to water. They were not fasted before the experiments. Ethanol was given s.c. in keeping with the same conditions as our previous experiments. The mice were killed by decapitation 1, 2 and 4 h respectively after the injection. The blood was collected in heparinized conical tubes and centrifuged for 10 min at average speed. The brain, heart, liver, uro-genital fat, kidneys and carcass were rapidly excised and immersed in saturated picric acid solution in previously weighed vials. After weighing, the tissues were transferred to microdistillation flasks and ethanol in each organ estimated according to LE BRETON et al.[6]. Control animals received identical treatment except that they were injected with NaCl only. Endogenous alcohol values were obtained by distilling 3 mice at a time. 3 determinations were made in each case. The CEO was obtained by estimating the alcohol in the distillate from a single mouse at a time. After injection, the animals were placed on a wire mesh resting on a glass trough and the whole covered by a large open bell-jar to minimize the loss by respiration. Urine and feces were quantitatively transferred to the distillation flask.

After an injection of 10 g/kg, the average ethanol concentration in all the organs studied was from 4–6 g/kg (Table I). The highest concentration was in the brain and the plasma. There was a progressive reduction in the concentration with time, the peak being obtained 1 h after the injection. There was a reduction of the wet weight of the brain, the heart and the liver (Table II). Endogenous alcohol had a value of 8.79 mg/kg. The CEO was $1578 \pm$

[1] H. M. THOMAS, J. TRÉMOLIÈRES, G. GRIFFATON and R. LOWY, Fd. Cosmet. Toxicol. *6*, 33 (1968).
[2] L. CARRÉ and J. TRÉMOLIÈRES, Bull. Soc. Chim. biol. *40*, 851 (1958).
[3] F. W. KINARD, Nature *200*, 852 (1963).
[4] E. MARSHALL and A. OWENS, Proc. Soc. exp. Biol. Med. *82*, 573 (1955).
[5] G. H. NELSON, F. W. KINARD and M. G. HAY, Am. J. Physiol. *190*, 169 (1957).
[6] E. LE BRETON, M. NICLOUX and L. DONTCHEFF, Bull. Soc. Chim. biol. *16*, 1314 (1934).