The Intracellular pH of the Isolated Perfused Rat Liver

DMO (dimethadione; 5,5-dimethyloxazolidine-2,4-dione) has been widely used to determine the intracellular pH of many tissues[1]. In these conditions, values for erythrocyte pH are approximately consistent with a Gibbs-Donnan equilibrium[2]. In contrast, estimates of muscle cell pH are about 1.0 pH units higher than the equilibrium value predicted from the membrane potential[3]. Since circumstantial evidence[1] suggests that hydrogen ions are passively distributed across cell membranes, the difference may be related to the presence of relatively alkaline mitochondria[4] in skeletal muscle cells. In the mammalian liver, mitochondria represent approximately 20% of the total intracellular volume; thus, the internal pH of the liver cell (as measured from the distribution of DMO) may well be higher than the value consistent with electrochemical equilibrium.

In the present work, intracellular pH was determined experimentally from the distribution of DMO-2-C^{14} in the isolated perfused rat liver. The results obtained were compared with equilibrium values for cell pH, which were calculated from published estimates of the potential difference across the liver cell membrane.

Male Wistar rats (300–320 g body weight) were anaesthetized with urethane (1.4 g/kg, i.p.). The isolated liver was perfused in situ[5] at 35 °C with an oxygenated (95% O_2: 5% CO_2) modified Krebs-Ringer bicarbonate buffer containing dextran 110 B.P. (6%) and chloramphenicol (0.25 m*M*). Rates of perfusion ranged from 3–5 ml/g liver/min. Perfusate pH was maintained at 7.40 ± 0.01 by the occasional addition of sodium bicarbonate solution (8.4% w/v).

At the start of each experiment DMO-2-C^{14} (1.0 µCi) and inulin-carboxyl-C^{14} (2.0 µCi) were added to the perfusion medium and allowed to equilibrate for 1 h. Duplicate weighed samples of liver were then homogenized with sodium dihydrogen phosphate solution (5 *M*; 1.0 ml) and DMO-2-C^{14} was extracted several times with an ethyl acetate-toluene mixture (50% v/v)[6]. DMO-2-C^{14} was similarly extracted from samples of the perfusate, and the radioactivity in the extracts was determined by liquid scintillation spectrometry[2]. Total radioactivity in weighed samples of liver and perfusate was measured by standard methods[7]; inulin-carboxyl-C^{14} was then determined by subtracting the values (dpm/g) previously obtained for DMO-2-C^{14}. Estimates of the extracellular space of the isolated rat liver, calculated from the distribution of inulin-carboxyl-C^{14}, were consistent with comparable measurements based on other techniques[8] (Table).

At the end of each experiment, part of the liver (usually the median lobe) was weighed separately and placed in a hot air oven (110 ± 10 °C). The loss of weight after drying was assumed to be equivalent to the total liver water.

The intracellular pH of the liver cell was calculated from a modification[2] of the equation of WADDELL and BUTLER[9]. At an external pH of 7.40 ± 0.01 and a pCO_2 of 35–37 mm Hg, the intracellular pH of the normal rat liver cell at 35 °C was 7.17 ± 0.09 (mean $\pm$ standard deviation). In 4 experiments, individual values for internal cell pH ranged from 7.09–7.30 (Table). Another study of the intracellular pH of the isolated perfused rat liver[10] was reported while this work was in progress; in these experiments, intracellular pH ranged from 6.90–7.16, and was independent of the external carbon dioxide concentration.

The potential difference across the liver cell membrane has been determined in several mammalian species under differing experimental conditions. In normal rat liver at 35 °C (i.e., the same temperature that was used in the present study), there is a mean intracellular potential of -28 mV[11]. The distribution of diffusable ions at equilibrium is related to the membrane potential by the expression $r = 1/e^{-FE/RT}$, where E is the membrane potential in volts, r is the ionic distribution ratio between the cell and extracellular fluid, and e, F, R, and T have their usual meanings[2]. Thus, if hydrogen ions are passively distributed across the liver cell membrane and the external pH is 7.40, a potential difference of -28 mV is consistent with an intracellular pH of 6.94. These calculations are dependent on the assumption that pH is a negative logarithmic function of hydrogen ion activity. The results of the present experiments suggest that the intracellular pH of the isolated perfused rat liver is approximately 0.2 pH units higher than the values consistent with electrochemical equilibrium. Although other explanations cannot be excluded, this difference may be related to the presence of mitochondria in the liver cell.

Zusammenfassung. Die intrazellulären pH-Werte von Rattenleber wurden mit Hilfe der DMO-Verteilungsmethode untersucht. Der intrazelluläre pH-Mittelwert der Leberzellen betrug $7{,}17 \pm 0{,}09$; die intra-extrazelluläre pH-Differenz war 0,23.

T. N. CALVEY

Department of Pharmacology and General Therapeutics, University of Liverpool, Liverpool L69 3BX (England),

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DMO-2-C^{14} in liver (dpm/g)	DMO-2-C^{14} in perfusate (dpm/ml)	Liver water		Cell pH
		Intra-cellular (ml/g)	Extra-cellular (ml/g)	
13,680	25,800	0.46	0.25	7.17
17,450	27,675	0.47	0.25	7.30
10,790	20,767	0.50	0.26	7.11
10,205	19,721	0.48	0.27	7.09

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