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Species differences of galactose metabolism in erythrocytes

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Galactose metabolism was studied in suspensions of erythrocytes from man, rat, ox and pig at approximate steady-state galactose concentrations from 0.1–6.5 mmol/l. There was no detectable galactose elimination in pig erythrocytes. In the three other species the elimination followed saturation kinetics with the maximal elimination rate, V_{max}, being 8.0 $\mu mol \cdot min^{-1} \cdot l^{-1}$ human erythrocytes; 12 $\mu mol \cdot min^{-1} \cdot l^{-1}$ rat erythrocytes; 2.4 $\mu mol \cdot min^{-1} \cdot l^{-1}$ bovine erythrocytes. The half saturation concentration, K_m, was about the same in all three species, around 0.2 mmol/l. If similar *in vivo*, erythrocyte metabolism of galactose is not of major quantitative importance compared to the hepatic metabolism in the species examined.

Key words: erythrocyte; galactose; ox; pig; rat

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Erythrocytes from various species show marked differences in utilization of glucose [1]. For example, pig erythrocytes have a glucose utilization rate that is less than one quarter of that in erythrocytes from man, ox or rat [2, 3] perhaps due to variations in membrane permeability [3]. Pig erythrocytes are unable to metabolize glucose *in vitro* in an amount sufficient to account for the energy need of the cells, and it has been suggested that pentoses or purine nucleosides may be important energy sources for pig erythrocytes [3].

Only few studies have been published on galactose metabolism in erythrocytes. It has been shown that human erythrocytes contain the enzymes necessary for galactose metabolism [4] and that they are able to metabolize galactose *in vitro* [5], that rat erythrocytes metabolize galactose at a rate about one quarter of that of glucose [6] and that there is no detectable galactose metabolism in pig erythrocytes [7].

Galactose is metabolized mainly in the liver, but in the present study we have examined galactose elimination in erythrocytes to evaluate possible quantitative importance *in vivo* in human subjects [8], rats [9], and pigs [10], and in experimentally perfused livers [7, 11]. Since the medium used for experimental perfusion of rat livers often contains bovine erythrocytes, galactose metabolism in erythrocytes from this source was also studied.

SUBJECTS AND METHODS

Erythrocyte suspensions. Human blood was obtained by vein puncture of healthy volunteers

(n=7), rat blood by puncture of the heart of 300 g Wistar rats under light aether anaesthesia (n=5) and pig blood from 40–50 kg pigs of Danish land race during anaesthesia with halothane, N_2O, and oxygen (0.8:66:33) (n=5); blood from these three sources was sampled in heparinized bottles and washed immediately (see below). Bovine blood was obtained from the slaughter house in connection with exsanguination of the animals (n=3), the blood was sampled in bottles with 125,000 IU heparin per 5 l of blood and kept at 4 °C until washing was performed the next day.

During the washing procedure the cells were washed four times with 0.9 g/100 ml NaCl followed by centrifugation, removal of the supernatant and filtration through gauze (Monedur, 45 μm, Square Compani, Copenhagen, Denmark).

The erythrocytes were suspended in a modified Krebs-Henseleit buffer (NaCl 137 mmol/l, KCl 2.68 mmol/l, $CaCl_2 \cdot H_2O$ 1.80 mmol/l, $MgCl_2 \cdot 6H_2O$ 0.49 mmol/l, $NaHCO_3$ 11.9 mmol/l and $NaH_2PO_4 \cdot H_2O$ 0.67 mmol/l) titrated to pH 7.4 with NaOH 1 mol/l and oxygenated with air:CO_2 (95:5). Bovine albumin (fraction V; ethanol-precipitated; Sigma Chemical Co., London, UK (with ethanol removed by distillation of the solution in vacuo at 40 °C for 5 h)) was added to the medium to a final concentration of 20 l/l. The haematocrit, measured in micro-capillary tubes after centrifugation in a Crist microhaematocrit centrifuge for 5 min, ranged from 0.25–0.44 g/l. The oxygen tension (Radiometer, Copenhagen, Denmark) was 150–210 mmHg in the beginning of the measurement period and 168–216 at the end, respectively; the oxygen saturation 0.98–0.99 throughout the measurement period.

Galactose metabolism. Galactose (Kabi, Stockholm, Sweden) was added to the erythrocyte suspensions so that concentrations from 0.1–6.5 mmol/l were obtained. The suspensions were kept in Erlenmeyer bottles at 37 °C in a shaking water-bath. Samples of the medium were taken every tenth minute in 90 min and analysed for galactose by an enzymatic method [12]. In control experiments without erythrocytes, galactose concentrations did not increase significantly in the course of the measurement period ($p > 0.5$), the measurement accuracy being 0.05 mmol/l, showing that there was no significant evaporation of water from the medium during the time course of the experimental period.

The galactose concentration-time curves were linear within the measurement precision, the elimination rate was calculated from the slope of the linear regression line. The maximal elimination rate V_{max}, and the half saturation concentration K_m, were calculated by weighed linear regression of the Lineweaver–Burk double reciprocal relation of elimination rate on mean concentration of galactose.

RESULTS

Figure 1 shows plots of the elimination rate against the mean concentrations of galactose

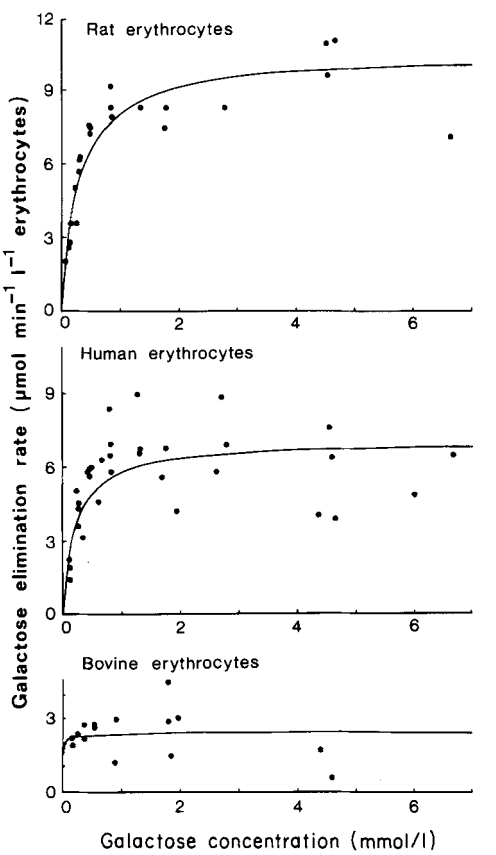

FIG. 1. Galactose steady-state metabolism in suspensions of erythrocytes from human subjects, rats, and oxen. The points give the experimental data and the curves the Michaelis–Menten relations calculated from estimated values of V_{max} and K_m (see text).

(there was no measurable galactose metabolism in the pig erythrocytes, the slope of the regression of concentration on time being not significantly different from zero ($p > 0.4$ in each experiment), data not shown in the figure). Figure 1 shows that in the erythrocytes from the other three species the metabolism followed saturation kinetics with the removal rate being highest in the rat erythrocytes.

There was no observable difference in the metabolism in erythrocytes from different subjects within each of the species, and therefore V_{max} and K_m were calculated as common estimates for each of the species. For the rat erythrocytes V_{max} was 12.0 $\mu mol \cdot min^{-1} \cdot l^{-1}$ erythrocytes$\pm$0.8 ($\pm$ standard error of the estimate) and K_m 0.30 mmol/l$\pm$0.05; for the human erythrocytes, V_{max} was 8.0 $\mu mol \cdot min^{-1} \cdot l^{-1} \pm 0.6$ and K_m 0.20 mmol/l$\pm$0.4. For the bovine erythrocytes V_{max} was 2.4 $\mu mol \cdot min^{-1} \cdot l^{-1} \pm 0.4$, whereas K_m could not be determined with reasonable accuracy although it is clear from the figure that it is below 0.2 mmol/l. The V_{max} values were statistically significantly different (analysis of variance, $p < 0.01$).

DISCUSSION

The study shows marked species differences in erythrocyte metabolism of galactose. The removal rate in the human erythrocytes is of the same order of magnitude as found by Henderson *et al.* [5], and the undetectable low rate in pig erythrocytes corresponds to our previous results [7]. During physiological conditions, blood galactose concentration is low, less than 0.1 mmol/l, and therefore galactose probably plays no role in the energy metabolism of the erythrocytes in the species examined here.

As seen on the figure the scatter of the data around the calculated Michaelis–Menten curve is large—and the estimated errors of the values of V_{max} and K_m are correspondingly large. On the other hand, there is no doubt that the elimination is saturated at concentrations higher than about 1 mmol/l. The figure could give the impression that the elimination rate decreases at very high concentrations; this could be due to substrate inhibition as it is found for the enzyme galactokinase from pig liver [13]. Application of this model to the data does not, however, give a significantly smaller residual variance of the analysis. Therefore the simple Michaelis–Menten model was chosen to describe the present relationship between elimination rate and concentration.

Rat livers which are perfused by a medium containing bovine erythrocytes, metabolize galactose at a rate of about 0.35 $\mu mol \cdot min^{-1} \cdot g^{-1}$ liver (at galactose concentrations higher than 2 mmol/l ensuring near-saturated elimination [11]). At a flow rate of the medium of 1.0 $ml \cdot min^{-1} \cdot g^{-1}$ liver with haematocrit 0.25 l/l, the calculated elimination in bovine erythrocytes will be about 0.0006 μmol galactose$\cdot min^{-1} \cdot g^{-1}$ liver. If rat erythrocytes are used in the medium, the total galactose elimination rate is somewhat higher, about 0.40 $\mu mol \cdot min^{-1} \cdot g^{-1}$ liver (S. Keiding, unpublished observations) and the calculated erythrocyte metabolism about 0.01 $\mu mol \cdot min^{-1} \cdot g^{-1}$ liver. Thus the usual procedure of not accounting for erythrocyte galactose elimination in perfused liver preparations seems justified.

The galactose transport across the membrane of the human erythrocytes has been described as a two-sided carrier-facilitated diffusion with apparent K_m values 20–50 mmol/l [6, 14, 15]. These K_m values are high compared to the present values for metabolism, indicating that metabolism will be saturated at concentrations at which membrane transport is concentration dependent.

If one assumes a similar galactose elimination rate in the erythrocytes in man *in vivo* and a total blood volume of 5 l with haematocrit 0.45 l/l, this would yield a rate of erythrocyte galactose elimination of approximately 0.02 mmol/min, at galactose concentrations higher than 2 mmol/l. This is not of quantitative importance *in vivo* where the total elimination is about 2.5 mmol/min in control subjects [8] and the minimum elimination during severe liver damage is about 0.5 mmol/min [16]. Thus, erythrocyte metabolism of galactose is probably not responsible for a possible extrahepatic galactose metabolism [17].

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REFERENCES

1 Laris PC. Permeability and utilization of glucose in mammalian erythrocytes. J Cell Comp Physiol 1958; 51: 273–307.

2 McManus TJ. Comparative biology of red cells. Fed Proc 1967; 26: 1821–1826.

3 McManus TJ, Kim H-D. Energy metabolism in the pig red cell. In: Deutsch E, Gerlach E, Moser K (eds). Metabolism and membrane permeability of erythrocytes and trombocytes. Stuttgart: Thieme, 1968: 43–50.

4 Isselbacher KJ. Galactosemia: Clinical Staff Conference at the National Institutes of Health. Ann Intern Med 1957; 46: 773–775.

5 Henderson JM, Fales FW. Continuous-flow fluorometry of low galactose concentrations in blood or plasma. Clin Chem 1980; 26: 2282–2285.

6 Miller DM. The kinetics of selective biological transport. I. Determination of transport constants for sugar movements in human erythrocytes. Biophys J 1965; 5: 407–423.

7 Keiding S, Johansen S, Winkler K, Tønnesen K, Tygstrup N. Michaelis-Menten kinetics of galactose elimination by the isolated perfused pig liver. Am J Physiol 1976; 320: 1302–1313.

8 Tygstrup N. Determination of the hepatic elimination capacity (Lm) of galactose by single injection. Scand J Clin Lab Invest 1966; 18: 118–125.

9 Keiding S. The galactose elimination capacity in the rat. Scand J Clin Lab Invest 1973; 31: 319–325.

10 Keiding S, Johansen S, Winkler K. Hepatic galactose elimination kinetics in the intact pig liver. Scand J Clin Lab Invest 1982; 42: 253–259.

11 Vilstrup H, Keiding S, Vendsborg P. Kinetics of galactose uptake by perfused rat livers: applicability of a family of models. J Theor Biol 1983; 101: 335–344.

12 Kurz G, Wallenfels K. D-galactose. UV-Test mit Galactosedehydrogenase. In: Bergmeyer HU, ed. Methoden der enzymatischen analyse. Weinheim: Verlag Chemie, 1970: 1241–1244.

13 Ballard FJ. Purification and properties of galactokinase from pig liver. Biochem J 1966; 98: 347–352.

14 Levine M, Stein WD. The kinetic parameters of the monosaccharide transfer system of the human erythrocytes. Biochim Biophys Acta 1966; 127: 179–193.

15 Ginsburg H, Stein WD. Zero-trans and infinite-cis uptake of galactose in human erythrocytes. Biochim Biophys Acta 1975; 382: 353–368.

16 Ranek L, Andreasen PB, Tygstrup N. Galactose elimination capacity as a prognostic index in patients with fulminant liver failure. *Gut* 1976; 17: 959–964.

17 Ranek L, Lindskov J, Tygstrup N, Winkler K. Splanchnic galactose uptake in patients with cirrhosis following single injection. Clin Physiol 1983; 3: 173–178.

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