*Biochem. J.* (1980) **192**, 373–375
*Printed in Great Britain*



# Transport of D-fructose and D-galactose into isolated rat hepatocytes

James D. CRAIK and Keith R. F. ELLIOTT
*Department of Biochemistry, University of Manchester, Oxford Road, Manchester M13 9PL, U.K.*

(*Received 2 July 1980/Accepted 22 July 1980*)

Transport of D-fructose and D-galactose across the plasma membrane of isolated rat hepatocytes was followed for the net entry of sugars into sugar-free cells at 20°C. Initial rates of transport showed a Michaelis–Menten dependency on sugar concentration, and transport was inhibited by 3-*O*-methyl-D-glucose in the external medium.

D-Fructose and D-galactose are rapidly cleared from the portal circulation on passage through the liver. Because of technical problems, it has been difficult to differentiate between cell membrane transport and intracellular metabolic sequestration by using the intact liver.

It is apparent that, in the dog (Goresky *et al.*, 1973) and pig (Keiding *et al.*, 1976), the membrane transport capacity for D-galactose is substantially higher than the metabolic capacity of the liver. However, perfusion studies with rat liver indicate that membrane transport can limit D-fructose metabolism under certain conditions (Woods *et al.*, 1970; Sestoft & Fleron, 1974).

An earlier study of monosaccharide transport into isolated hepatocytes showed no saturation of D-fructose transport (at 20°C) up to a concentration of 100mM (Baur & Heldt, 1977), in contrast with a $K_m$ of 67mM derived from perfusion studies (Sestoft & Fleron, 1974). D-Galactose appeared to be transported into the isolated hepatocytes by an equilibrative system with a $K_m$ of 100mM (Baur & Heldt, 1977).

Here we report the kinetic parameters for transport of D-fructose and D-galactose into isolated hepatocytes, obtained by using methods previously used to study 3-*O*-methyl-D-glucose transport in detail (Craik & Elliott, 1979).

## Materials and methods

Male Sprague–Dawley rats (250–350g) were deprived of food for 24 or 48h before cell preparation.

3-*O*-Methyl-D-glucose, D-fructose, D-galactose and phloretin were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. Collagenase was from Boehringer Corp. (London) Ltd., Lewes, East Sussex, U.K.

[U-$^{14}$C]3-*O*-Methyl-D-glucose (sp. radioactivity 74.2mCi/mmol), D-[U-$^{14}$C]fructose (sp. radioactivity 241mCi/mmol) and D-[1-$^{3}$H]galactose (sp. radioactivity 22.0Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

All other chemicals were of the purest grade available from standard suppliers.

Centrifugation steps were performed with an MSE Super Minor bench centrifuge with a swing-out head. All transport experiments were performed in 100mm × 13mm plastic tubes.

### *Cell preparation*

Isolated rat hepatocytes were prepared by the collagenase perfusion method (Berry & Friend, 1969) as described in detail previously (Craik & Elliott, 1979; Elliott *et al.*, 1976).

### *Transport measurements*

The entry of D-fructose and D-galactose into extensively washed hepatic parenchymal cells was followed by the method detailed for 3-*O*-methyl-D-glucose entry (Craik & Elliott, 1979).

Progress curves were defined by 16 to 28 experimental time points taken over 50 or 60s, most points being taken between 2.5 and 20s of incubation. Initial rates were estimated as tangents by eye to progress curves at zero time.

Osmotic effects were minimized by compensatory changes in the NaCl concentration in the phosphate–Ringer buffer for sugar concentrations from 30 to 100mM, and by dilution of the total buffer at higher sugar concentrations.

In competition experiments the same methodology was followed with the addition of an appropriate concentration of competing sugar in the external medium.

### *Termination of transport*

The cold (1–2°C) stopper solution [300$\mu$M-phloretin, 0.7% (v/v) ethanol, 80$\mu$M-$HgCl_2$, 50$\mu$M-KI in 1% (w/v) NaCl], known to be an effective



0306-3283/80/100373-03$01.50/1 

inhibitor of 3-*O*-methyl-D-glucose transport (Craik & Elliott, 1979), was used.

Sugar uptake in the presence of the stopper solution at 20°C was less than 3% of the uninhibited rate and less than 0.5% at 1–2°C. Zero-time values obtained as described previously (Craik & Elliott, 1979) indicated that the onset of inhibition was very rapid in comparison with entry fluxes.

*Water spaces*

Intracellular water spaces were routinely determined with 5mM- or 10mM-3-*O*-methyl-D-glucose as reported previously (Craik & Elliott, 1979).

**Results and discussion**

Fig. 1 shows typical progress curves for D-fructose transport into hepatocytes at low (8.8mM) and high (153mM) concentrations. At the low concentration the curve is close to being linear, suggesting a relatively rapid rate of metabolism in the cell. However, at higher concentrations the curves resemble those obtained for the zero-*trans* entry of the non-metabolizable 3-*O*-methyl-D-glucose (Craik & Elliott, 1979), indicating that transport exceeds metabolism. Progress curves for D-galactose were qualitatively similar.

Logarithmic plots of initial rate of sugar transport against external sugar concentration are shown in Fig. 2. The results are consistent with a single carrier showing a Michaelis–Menten relationship between transport rate and sugar concentration. Kinetic constants were estimated from the data by the method of Wilkinson (1961) as: $V_{max.}$ 291 ± 26 mmol/litre of cell water per min and $K_m$ 212 ± 32 mM for D-fructose (mean ± S.E.M., $n = 38$); and $V_{max.}$ 288 ± 48 mmol/litre of cell water per min and $K_m$ 174 ± 48 mM for D-galactose (mean ± S.E.M., $n = 24$). At sugar concentrations substantially below the apparent $K_m$ for transport, the data points lie along a slope of unit gradient, showing no evidence of polyvalency of the system towards either external D-fructose or D-galactose. These results are qualitatively similar to those obtained for zero-*trans* entry of 3-*O*-methyl-D-glucose (Craik & Elliott, 1979).

Entry of both D-fructose and D-galactose is readily inhibited by the presence of external 3-*O*-methyl-D-glucose, with a $K_i$ of 27 and 30mM respectively (cf. $K_m$ for 3-*O*-methyl-D-glucose transport of 20mM; Craik & Elliott, 1979). There was also mutual inhibition shown between D-fructose and

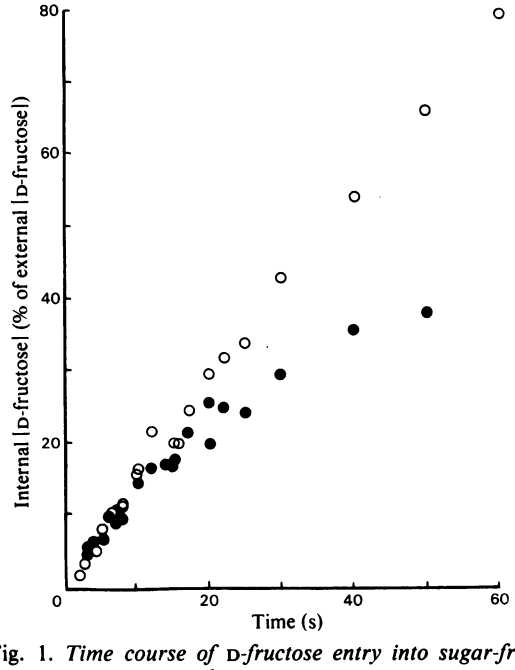


Fig. 1. *Time course of D-fructose entry into sugar-free hepatocytes*

The incubations were performed at 20°C and pH 7.4 in phosphate–Ringer buffer as described in the Materials and methods section. External D-fructose concentrations at zero time were: ○, 8.8mM; ●, 153mM.

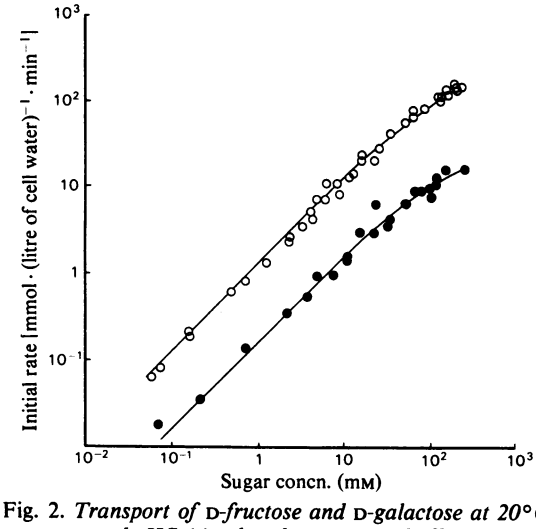


Fig. 2. *Transport of D-fructose and D-galactose at 20°C and pH 7.4 in phosphate–Ringer buffer*

The incubations were performed as described in the Materials and methods section. The solid lines represent the Michaelis–Menten curves for $V_{max.}$ 291 mmol/litre of cell water per min and $K_m$ 212mM for D-fructose, and $V_{max.}$ 288 mmol/litre of cell water per min and $K_m$ 174mM for D-galactose. Symbols: ○, D-fructose; ●, D-galactose. The data points and curve for D-galactose have been displaced on the ordinate by $10^{-1}$ for clarity.

D-galactose. The apparent $K_i$ values, consistent with competitive inhibition, were 210mM for D-galactose and 230mM for D-fructose. The apparent competitive nature of the inhibition and the similarities of the $K_i$ and $K_m$ values indicate that the three sugars are probably being transported into the hepatocytes by a single system.

The kinetic parameters obtained for D-fructose and D-galactose transport into the isolated hepatocytes show some similarities to those obtained previously. The reasons for obtaining higher values than those of Baur & Heldt (1977), who also used isolated hepatocytes, have been discussed elsewhere (Craik & Elliott, 1979). Unlike Baur & Heldt (1977), we have been able to show saturation kinetics for D-fructose transport.

From the kinetic parameters obtained, it can be seen that at low sugar concentrations D-galactose is transported more rapidly than D-fructose, and entry of both sugars is inhibited by external 3-*O*-methyl-D-glucose. This is in agreement with results obtained from liver-perfusion studies in sheep (Hooper & Short, 1977) and dogs (Goresky *et al.*, 1973; Goresky & Nadeau, 1974). The $K_m$ values obtained (at 20°C) are, however, higher than that of 30mM for D-galactose transport into perfused dog liver (Goresky *et al.*, 1973) and 67mM for D-fructose transport into perfused rat liver (Sestoft & Fleron, 1974) at physiological temperature. They are also substantially higher than the half-maximal concentration for clearence of D-galactose by the perfused pig liver (Keiding *et al.*, 1976). These differences are probably due to the vastly different methodologies employed. In particular, the clearance studies do not separate transport from metabolism, whereas here we are reporting rates of transport obtained by direct measurement.

This work was financed by a grant from the Medical Research Council.

## References

Baur, H. & Heldt, H. W. (1977) *Eur. J. Biochem.* **74**, 397–403

Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520

Craik, J. D. & Elliott, K. R. F. (1979) *Biochem. J.* **182**, 503–508

Elliott, K. R. F., Ash, R., Pogson, C. I., Smith, S. A. & Crisp, D. M. (1976) in *Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies* (Tager, J. M., Söling, H.-D. & Williamson, J. R., eds.), pp. 139–143, North-Holland, Amsterdam

Goresky, C. A. & Nadeau, B. E. (1974) *J. Clin. Invest.* **53**, 634–646

Goresky, C. A., Bach, C. G. & Nadeau, B. E. (1973) *J. Clin. Invest.* **52**, 991–1009

Hooper, R. H. & Short, A. H. (1977) *J. Physiol. (London)* **264**, 523–539

Keiding, S., Johansen, S., Winkler, K., Tønnesen, K. & Tygstrup, N. (1976) *Am. J. Physiol.* **230**, 1302–1313

Sestoft, L. & Fleron, P. (1974) *Biochim. Biophys. Acta* **345**, 27–38

Wilkinson, G. N. (1961) *Biochem. J.* **80**, 324–332

Woods, H. F., Eggleston, L. V. & Krebs, H. A. (1970) *Biochem. J.* **119**, 501–510