

## CARTA AL EDITOR

## Effect of Phlorizin and Some Phenylgalactosides on the Active Transport of D-galactose by Intestinal Rings of Hamster

ALVARADO and CRANE (1) observed that phlorizin was a competitive inhibitor of the active transport of 1,5-anhydroglucitol and 6-deoxyglucose by hamster small intestine. The same authors have also studied (2) the inhibitory action of other phenylglycosides on the active transport of sugars and its possible relationship to phlorizin inhibition, suggesting that certain structural requirement in the phenylglycoside series would be essential for interaction with the active transport process. In this respect, it was of interest to test the action of some phenylgalactosides and phlorizin on the active transport of D-galactose.

Golden hamsters (*Mesocricetus auratus*), 80-110 g body weight, were used. Everted intestinal rings were obtained by the CRANE and MANDELSTAM technique (4), and were placed in 4 ml of Krebs-Henseleit bicarbonate buffer (5), with the appropriate added substrate. The medium were bubbled with carbogen during the whole incubation period. Data were processed as previously described (3). The inert sugar was determined by the Somogyi method (6) and D-[1-<sup>14</sup>C] galactose in a liquid scintillation counter (toluene/ethanol scintillation mixture was used).

Table I indicates that phlorizin, at  $10^{-4}$  M to  $10^{-6}$  M concentrations in the

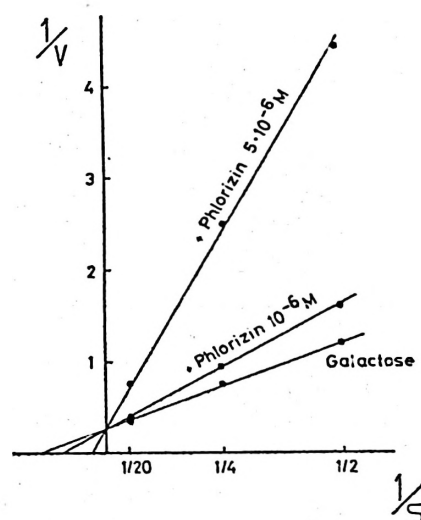


Fig. 1. Inhibition by phlorizin of the active transport of D-galactose by rings of hamster small intestine in vitro.

Incubation time, 10 min. The initial sugar concentration were 20, 4 and 2 mM. The individual points are the averages of 18 (galactose only), 8 (plus  $10^{-6}$  M phlorizin) and 10 (plus  $5 \times 10^{-6}$  M phlorizin) experiments.

medium, inhibits the active accumulation of galactose. This inhibition, as deduced from the figure 1, is clearly competitive with the D-galactose, yielding an apparent  $K_i$  for phlorizin of  $1.5$  to  $2 \times 10^{-6}$  M. This value is close to that found by AL-

Table 1. *Effect of phlorizin and some phenylgalactosides on the accumulation of D-galactose by rings of hamster small intestine.*

Incubations were for 20 minutes in 4 ml medium. Initial substrate and inhibitor concentrations, as indicated. The data are corrected for D-manose space and accompanied by the average standard error. The number of experiments is given in parentheses.

Substrate	Inhibitor	[M]	Final concentration of galactose (mM)		Inhibition %
			Tissue	Medium	
Galactose (4 mM)	No addition		25.90 ± 0.40	2.56 ± 0.06 (86)	
	Phlorizin	10 <sup>-6</sup>	16.26 ± 0.62	3.22 ± 0.13 (9)	33.93
	"	5 × 10 <sup>-6</sup>	10.17 ± 0.57	3.54 ± 0.11 (9)	58.91
	"	10 <sup>-5</sup>	7.25 ± 0.35	3.81 ± 0.10 (12)	70.11
	"	10 <sup>-4</sup>	1.77 ± 0.19	4.00 ± 0.00 (15)	93.16
	4-nitrophenylgalactoside	10 <sup>-4</sup>	26.06 ± 1.39	2.52 ± 0.12 (6)	—
	"	5 × 10 <sup>-4</sup>	25.51 ± 1.30	2.59 ± 0.15 (18)	—
	"	10 <sup>-3</sup>	23.05 ± 1.10	2.71 ± 0.11 (12)	—
	"	5 × 10 <sup>-3</sup>	24.19 ± 1.07	2.65 ± 0.13 (14)	—
	2-nitrophenylgalactoside	10 <sup>-3</sup>	24.50 ± 0.71	2.70 ± 0.15 (7)	—
	"	5 × 10 <sup>-3</sup>	23.56 ± 1.23	2.76 ± 0.18 (12)	—
Galactose [1- <sup>14</sup> C] (4 mM)	No addition		22.14 ± 1.40	3.06 ± 0.10 (6)	
	Phenylgalactoside	5 × 10 <sup>-3</sup>	18.22 ± 1.07	3.24 ± 0.08 (9)	17.71
	4-nitrophenylgalactoside	5 × 10 <sup>-3</sup>	22.48 ± 0.95	3.10 ± 0.12 (4)	—
Galactose [1- <sup>14</sup> C] (1 mM)	No addition		6.09 ± 0.23	0.78 ± 0.02 (13)	
	Phenylgalactoside	5 × 10 <sup>-3</sup>	4.45 ± 0.11	0.82 ± 0.01 (7)	26.93
	4-nitrophenylgalactoside	5 × 10 <sup>-3</sup>	5.92 ± 0.14	0.80 ± 0.02 (9)	—
	2-nitrophenylgalactoside	5 × 10 <sup>-3</sup>	5.87 ± 0.15	0.80 ± 0.01 (6)	—

VARADO and CRANE (1) when 6-deoxyglucose and 1,5-anhydroglucitol were used.

The addition of 2- or 4-nitrophenyl-β-D-galactopyranosides instead of phlorizin, did not affect the transport of galactose even at 5 × 10<sup>-3</sup> M concentration. However, 5 × 10<sup>-3</sup> phenylgalactoside shows a weak inhibitory action which is more clearly observed with low levels of galactose (1 mM). [1-<sup>14</sup>C] galactose was used in these experiments to avoid eventual interferences.

The results reveal that addition of the phenyl group to the galactose strongly decreases its affinity for the transport system, and if a NO<sub>2</sub> group is added further to the phenyl ring the affinity is entirely lost. This effects are similar to those found with phenyl- and nitrophenylglucosides on the transport of other sugars (2).

## References

1. ALVARADO, F. and CRANE, R. K.: *Biochim. Biophys. Acta*, **56**, 170, 1962.
2. ALVARADO, F. and CRANE, R. K.: *Biochim. Biophys. Acta*, **93**, 116, 1964.
3. BOLUFER, J., LARRALDE, J. and PONZ, F.: *Pflügers Arch. Eur. J. Physiol.*, **338**, 159, 1973.
4. CRANE, R. K. and MANDELSTAM, P.: *Biochim. Biophys. Acta*, **45**, 460, 1960.
5. KREBS, H. A. and HENSELEIT, K.: *Hoppe-Seyler's Z. Physiol. Chem.*, **210**, 33, 1932.
6. SOMOGYI, M.: *J. Biol. Chem.*, **195**, 19, 1952.

J. BOLUFER, J. LARRALDE and F. PONZ

Departamento de Investigaciones  
Fisiológicas (C.S.I.C.)  
Universidad de Navarra  
Pamplona (España)

(Received on 4 April, 1974)