(= 14,2%) and CSF osmolarity by 31.6 mosm/l (= 13%).

The development of osmolarity was very mild between day 11 and 13: by 3.2 mosm/l for CSF and by 3.0 mosm/l for blood plasma. The increase on day 15 was only significant ($p < 0.01$): 7.6 mosm/l for CSF, 8.9 mosm/l for blood plasma. A further significant elevation occurred between day 15 and 19 of incubation. The osmolarity increased in CSF by 17.4 mosm/l (= 6.8%) and by 23.0 mosm/l (= 8.9%) in blood plasma. The final developmental increase till the first posthatching day was more moderate and non-significant: in CSF by 6.9 mosm/l (= 2.5%), in blood plasma by 2.5 mosm/l (= 1.2%).

The osmolarity of both these fluids was identical till day 15 of incubation. The difference of 2.9–4.4 mosm/l and the plasma/CSF ratio of 1.012–1.017 were not significant. The osmolarity of blood plasma was regularly higher than the osmolarity of CSF only from day 17 of incubation. On day 17 the plasma-CSF difference increased to 8.6 mosm/l and the plasma/CSF ratio to 1.033. The maximum difference (10.0 mosm/l) and plasma/CSF ratio (1.037) was reached on day 19 of incubation. The values of both these parameters slightly decreased before and after hatching at the steady hyperosmolarity of blood plasma in comparison with the CSF.

Discussion. The direct measurements of plasma and CSF osmolarity did not confirm our previous theoretical presumption on the hyperosmolarity of CSF, which was calculated from the concentration of some ions, glucose and total proteins[3].

The developmental increase of the CSF and plasma osmolarity was parallel till day 19 of incubation. The increase of plasma osmolarity before and after hatching was smaller than in the CSF, which resulted in the anomaly in comparison with the development before day 19 of incubation. The development of chemical composition of the CSF and blood plasma[2,3], and especially the above-mentioned fact, showed that there probably was a barrier developing after day 15 of incubation, which ensured the difference between the concentration of osmotically active components in the CSF and blood plasma and which was responsible for a different development of osmolarity in both fluids, especially during the last days of incubation and after hatching. Consequently, the osmotic gradient from the blood plasma into the CSF must be taken into account since day 15 of incubation among the factors influencing the CSF formation and regulation of the CSF volume in the cerebral ventricular system[5] during this stage of the ontogenetic development.

Résumé. La pression osmotique du plasma sanguin et du fluide cérébrospinal (CSF) a été mesurée chez les embryons de poulet entre le 11e jour d'incubation et l'éclosion, et chez les poussins d'un jour. La pression osmotique augmente de 248,1 à 283,5 mosm/l dans le plasma sanguin et de 245,0 à 276,9 mosm/l dans le CSF. Dès le 11e jour du développement le plasma sanguin est hyperosmotique par rapport au CSF, ce qui prouve l'établissement d'une barrière osmotique entre le plasma sanguin et le CSF.

J. Sedláček

Research Laboratory of Psychiatry, Charles University,
Albertov 5, 128 00 Praha (Czechoslovakia),
12 May 1975.

---

[5] H. Davson, A Ciba Found. Symposium on CSF 1958, p. 189.

Interaction Between Sugars and Amino Acids in Intestinal Absorption by Rat, in vivo

Interaction between sugars and amino acids at intestinal level has received much attention in recent years. Cividanes et al.[1] showed that glucose and glycine absorption by rat intestine in vivo exhibit a mutual inhibition, but Bingham et al.[2] did not find any effect on amino acid absorption when glucose was present. More recently, Cooke[3] reported interaction between these substrates in man, and Bolufer et al.[4] have referred to the effect of leucine, glycine and arginine on glucose and galactose absorption by rat intestine, always on in vivo experiments. In the present paper, the mutual interaction between glucose, galactose and leucine absorption by the rat small intestine is studied in vivo.

Methods. Wistar rats of either sex of 150–200 g body weight were used. The animals were starved for 24 h

---

[1] L. Cividanes, J. Larralde and J. Bello, Revta esp. Fisiol. *20*, 11 (1964).

[2] J. K. Bingham, H. Newey and D. H. Smyth, Biochim. biophys. Acta *130*, 281 (1966).

[3] G. C. Cooke, J. Physiol., Lond. *217*, 61 (1971).

[4] J. Bolufer, J. Larralde and F. Ponz, Revta esp. Fisiol. *30*, 111 (1974).

L-leucine absorption by small intestine of rat in vivo at various initial concentrations

L-leucine (m*M*)	Absorption (µmol leucine/cm intestine)				
	1st	2nd	3rd	4th	5th
1[a] (4)	0.21 ± 0.02	0.20 ± 0.02	0.22 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
4[a] (4)	0.80 ± 0.01	0.78 ± 0.12	0.78 ± 0.14	0.84 ± 0.10	0.81 ± 0.11
20[a] (5)	2.91 ± 0.13	2.83 ± 0.08	2.87 ± 0.08	2.78 ± 0.12	2.80 ± 0.09
1 (6)	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.00	0.08 ± 0.01
4 (4)	0.43 ± 0.05	0.41 ± 0.04	0.40 ± 0.04	0.41 ± 0.06	0.42 ± 0.05
20 (6)	1.55 ± 0.10	1.50 ± 0.08	1.53 ± 0.09	1.57 ± 0.12	1.51 ± 0.07

The solution was recycled by peristaltic pumping or left in the intestinal loop. Each absorption period was of 5 min. Number of animals is given in brackets. The data are the mean ± SE. [a] With peristaltic pumping of 10 ml of solution with the appropriate added substrate through a closed circuit at a 13.54 ml/min rate.

before the experiment and anaesthetized with 12.5% urethane. The SOLS and PONZ[5] technique with or without a peristaltic pump to circulate the solution through the intestine was used. In perfusion experiments, a volume of 10 ml solution was pumped at 13.54 ml/min rate in a closed circuit through a segment of small intestine for 5 min and the corrections for water absorption were made. Sugars and amino acids were dissolved in a 0.9% NaCl solution, making corrections for constant osmolarity. The intestinal loop was rinsed with saline before and after each absorption period. 5 succesive periods of 5 min absorption were carried out on each animal. D-(1-^{14}C) galactose, D-(1-^{14}C) glucose and L-(1-^{14}C) leucine readings were taken in a liquid scintillation counter (a toluene/ethanol scintillation mixture was used). Results are expressed as µmoles of substrate absorbed per centimeter of intestine in 5 min.

Results and discussion. The normal values of intestinal absorption for L-leucine with or without peristaltic pumping are expressed in the Table. The rate of leucine absorption was higher when the solution was continually circulating in the system than when there was no pumping, probably because the substrate concentration in the intestinal loop remained higher throughout the absorption period on perfussion. With smaller initial concentrations of leucine, greater differences between both experimental conditions were found.

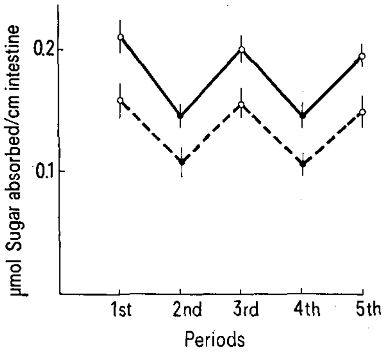

Fig. 1. Effect of 20 mM L-leucine on the intestinal absorption of D-glucose (——) and D-galactose (– – – –) at 1 mM initial concentration. Each period lasted 5 min. In the 2nd and 4th periods the amino acid was present. Results in µmol of sugar absorbed per cm intestine/5 min. The points are the average of experiments with 6 (glucose) or 5 (galactose) animals. Their ± SE is indicated.

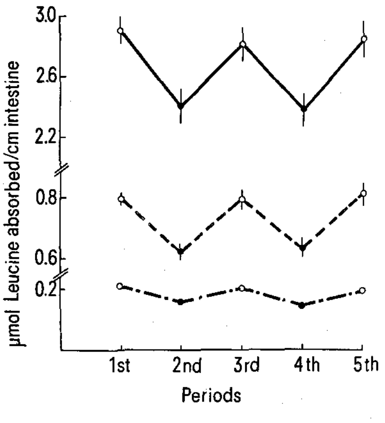

Fig. 2. Effect of 20 mM D-galactose on the intestinal absorption of L-leucine at 20 mM (——), 4 mM (– – – –) or 1 mM (–.–.–.–) initial concentrations. The sugar was present in the 2nd and 4th periods. Other conditions as in Figure 1.

The effect of 20 mM leucine on the intestinal absorption of 1 mM glucose or galactose is given in Figure 1. In the 2nd and 4th periods, when the amino acid was present, an inhibition of 33% for glucose and of 28% for galactose absorption was observed. This inhibition was reversible since in the 3rd and 5th periods when the leucine was omitted from the solutions; the normal values of sugar absorption were restored. The effect of 20 mM galactose on the intestinal absorption of 1, 4 or 20 mM leucine was also tested. The sugar was present during the 2nd and 4th periods. When the solution did not circulate through the intestine, only a slight inhibition of scant significance was observed. However, on closed circuit perfusion (Figure 2), with higher control values of leucine absorption, a clear inhibition was shown by the presence of galactose. This effect disappeared on changing the solution to one without galactose, as it occurred in the 3rd and 5th periods, when the values of leucine absorption were again normal. The percent differences between the 1st and 2nd periods without or with galactose were approximately 17%, 23% and 25% at 20,4 and 1 mM leucine initial concentration respectively.

Various hypotheses have been proposed to explain the mutual interactions between sugars and amino acids in intestinal absorption. As some researchers[2,6] failed to obtain any effect of galactose on amino acid uptake by the rat in vivo, they speculated that the inhibition exerted by galactose on in vitro experiments might possibly be due to competition for a common source of energy, possibly ATP. However, a mutual interaction between leucine and galactose in vivo with short absorption periods (5 min) has been proved in a previous[4] and in the present papers. Thus, the possible competition for energy is not in line with these findings, since in vivo the blood supplied enough glucose to provide energy.

MUNCK[7] observed that the inhibitory effect of galactose on intestinal amino acid transport by rat intestine in vitro results from a galactose-induced stimulation of amino acid efflux across the membrane of the epithelial cell, perhaps as result of the galactose-induced increase in tissue sodium concentration[8,9] and decrease in potassium concentration[10]. Though these changes have not been detected in the intracellular sodium concentration[9], this could be because only a very limited area of the brush border region is affected[8].

Stimulation of leucine efflux across the serosal face of the epithelial cells caused by the presence of galactose, should not inhibit but actually facilitate the amino acid absorption in vivo, whereas this does not in fact occur. However, stimulation of leucine efflux across the brush border membrane would be more in accordance with the results.

ALVARADO[11] explained this interaction with his hypothesis of a polyfunctional carrier, dependent on sodium, common to the intestinal transport of sugars and amino

---

[5] A. SOLS and F. PONZ, Revta esp. Fisiol. *3*, 207 (1947).

[6] J. K. BINGHAM, H. NEWEY and D. H. SMYTH, Biochim. biophys. Acta *120*, 314 (1966).

[7] B. G. MUNCK, Biochim. biophys. Acta *200*, 639 (1972).

[8] G. SEMENZA, Biochim. biophys. Acta *241*, 637 (1971).

[9] W. KOOPMAN and S. G. SCHULTZ, Biochim. biophys. Acta *173*, 338 (1969).

[10] M. M. BROWN and D. S. PARSONS, Biochim. biophys. Acta *59*, 249 (1962).

[11] F. ALVARADO, Science *151*, 1010 (1966).

acids. This view has recently been sustained[12–14]. An inhibitory allosteric effect, due to binding of the sugar sufficiently close to the amino acid-binding site or vice-versa, was suggested. Given the characteristics of the experimental technique used in the research described here, it is impossible to decide from the results which of the varying interpretations offered to explain this phenomenon is the most acceptable, even though the mutual interaction between galactose and leucine in vivo is very clearly confirmed.

Summary. The inhibitory action of L-leucine on the intestinal absorption of D-glucose and D-galactose, as well as the inhibitory action of D-galactose on the absorption of L-leucine at various concentrations by rat small intestine has been studied. The further effect was more clearly evidenced when the medium was perfunded through the intestine in a closed circuit system using a peristaltic pump.

J. Bolufer, J. Larralde and F. Ponz

Department of Animal Physiology,
University of Navarra, Pamplona (Spain),
16 April 1975.

---

[12] J. W. L. Robinson and F. Alvarado, Pflügers Arch. *326*, 48 (1971).

[13] F. Alvarado, R. Torres-Pinedo, L. Mateu and J. W. L. Robinson, FEBS Lett. *8*, 153 (1970).

[14] F. Alvarado and A. Mahmood, Biochemistry *13*, 2882 (1974).

The Effects of Jaundiced Plasma and Hypercholesterolaemic Plasma on Vascular Sensitivity to Injected Noradrenaline

Alterations in renal perfusion have been shown in a variety of liver diseases[1,2]. We have investigated the possibility that this may be due to a potentiated pressor response to circulating noradrenaline (NA).

Isolated perfused kidneys and segments of rabbit femoral artery were removed from an anaesthetized animal and perfused at constant flow in a warmed organ bath. The preparations wer first perfused with a physiological saline solution containing NaCl 118.0, KCl 4.69, NaH_2PO_4 1.33, $NaHCO_3$ 25.0, glucose 5.56, $CaCl_2$ 2.52 and $MgCl_2$ 1.05 *mM*/l and the perfusion pressure was monitored proximal to the preparation. As the flow was constant (4–5 ml/min), any change in resistance of the system was reflected in a change in perfusion pressure.

The arterial constrictor responses to NA were determined by graded injection of NA in warmed saline as a bolus into the perfusate just proximal to the preparation. These responses were obtained before, during and after perfusion of the preparation with the experimental plasma.

Figure 1 shows a log dose/response plot for a typical experiment when jaundiced baboon plasma was used. It can be seen that the jaundiced plasma caused the dose/response curve to shift to the left of the initial curve

---

[1] M. C. Kew, Gut *13*, 748 (1972).

[2] M. C. Kew, C. Limbrink, R. R. Varma and S. Sherlock, Gut *13*, 421 (1972).

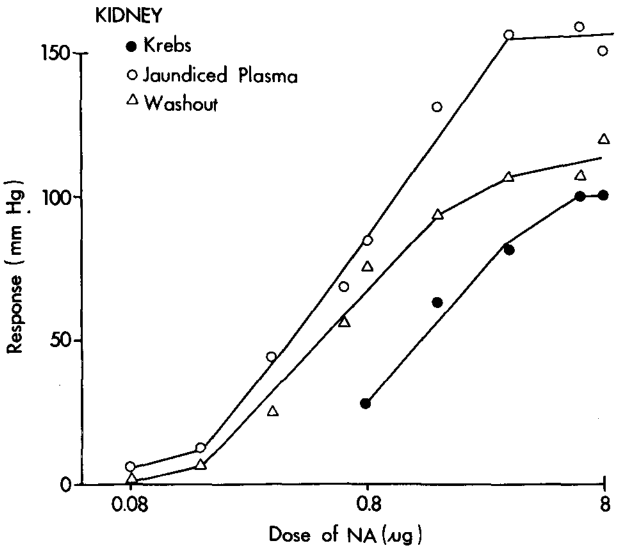

Fig. 1. Kidney log dose/responses – jaundiced plasma. Kidney responses in mm Hg (y axis) plotted against the doses of injected NA (x axis). The closed circles show the responses to NA obtained when Krebs was initially perfused. The open circles show those obtained when the jaundiced plasma was perfused, and the open triangles show those when Krebs was re-perfused. It can be seen that jaundiced plasma shifts the curve to the left and that the washout returns the curve towards normal.

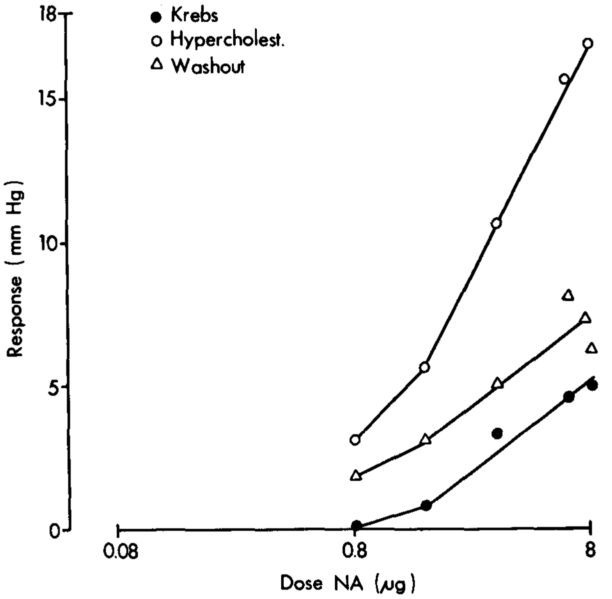

Fig. 2. Artery log dose/responses – lipid plasma. This graph shows the artery responses in mm Hg (y axis) plotted against the doses of injected NA (x axis). The closed circles show the responses obtained to NA when Krebs was initially perfused. The open circles show those obtained when the hypercholesterolaemic plasma was perfused, and the open triangles show those when Krebs was re-perfused. It can be seen that the plasma shifted the curve to the left and the washout moves back towards normal.