# A DISCREPANCY BETWEEN THE ACCUMULATION OF L-PHENYLALANINE IN THE INTESTINAL WALL AND THE APPEARANCE RATE IN THE BLOOD

D. WINNE

*Pharmakologisches Institut der Universität, D-7400 Tübingen, Germany*

Received 2 August 1972

## 1. Introduction

Generally the intestinal absorption of amino acids *in vivo* has been investigated by measuring the rate of disappearance from the intestinal lumen. As known to the author, only two papers [1, 2] have reported experiments where the appearance rate of some amino acids and dipeptides in the intestinal venous blood of cats and dogs were determined. In the present study results are reported which show a discrepancy between the accumulation of L-phenylalanine in the intestinal wall of rats and the appearance rate in the intestinal venous blood.

## 2. Experimental

Jejunal loops (5–8 cm) of urethane anaesthetized rats were perfused (single pass) with an isotonic buffered solution ($NaCl$-$KH_2PO_4$-$Na_2HPO_4$, pH 6.8) containing L-[U-$^{14}$C]phenylalanine and unlabelled L-phenylalanine, concentration 31.1 $\mu$M and 33.3 mM. The vascular connections to the neighbouring loops were interrupted. The draining vein of the loop was punctured and the blood was collected in a beaker. Since the collected blood was not reinfused, the loss of blood was compensated by an infusion of heparinized blood of rats. So a constant intestinal blood flow could be maintained. The venous blood and the perfusate were sampled every 15 min starting 10 min after the beginning of the perfusion. Half the experiments were stopped after the first period (25 min after starting the perfusion) and the concentration of L-phenylalanine was measured in the intestinal wall. The other experiments lasted 70 min (4 periods). From the weight of the collected blood the blood flow was calculated and related to wet tissue weight. The disappearance rate from the intestinal lumen was computed from the concentration difference of the luminal inflow and outflow solution, taking into consideration the water net flux determined in parallel experiments with polyethylene-1,2-[$^{14}$C]glycol. The appearance rate in the intestinal venous blood was derived from the blood flow and the concentration in the collected blood. For methodical details see Winne and Remischovsky [3].

## 3. Results and discussion

Using the solution with 31.1 $\mu$M L-phenylalanine, the absorption during the single pass perfusion of the jejunal loop reduced the concentration in the perfusate to 54% in the first and to 72% in the fourth period (see table). The concentration in the intestinal wall showed comparable values: 60% after the first and 75% after the last period. The concentration in the venous blood was 5–7% of that of the perfusion solution. The appearance rate in the intestinal blood was about 30% smaller than the disappearance rate from the intestinal lumen.

Using the solution with a 1000-fold higher concentration of L-phenylalanine (33.3 mM), the relative concentration in the intestinal wall was considerably lower (about 12%) indicating saturation of the accumulation mechanism. On the other hand, the concentration in the blood showed the same relation to the concentration in the perfusion solution as in the experiments with the low L-phenylalanine concentration: 5–6%. Thus the 1000-fold increase in the luminal



*North-Holland Publishing Company – Amsterdam*

Table 1
*In vivo* absorption of L-phenylalanine during single pass perfusion of a rat jejunal loop.

| Concentration perfusion solution (nmole $ml^{-1}$) | Time after starting the perfusion (min) | Number | Concentration perfusate (nmole $ml^{-1}$) | Intestinal venous blood (nmole $ml^{-1}$) | Intestinal wall (nmole $g^{-1}$) | Intestinal blood flow (ml $min^{-1}$ $g^{-1}$) | Disappearance from intestinal lumen (nmole $min^{-1}g^{-1}$) | Appearance in intestinal blood (nmole $min^{-1}g^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | 10–25 | 12 | 16.8 ± 0.6 | 2.2 ± 0.1 | | 0.86 ± 0.03 | 2.8 ± 0.2 | 1.9 ± 0.1 |
| | 25 | 6 | | | 18.7 ± 1.8 | | | |
| 31.1 | 25–40 | 6 | 19.0 ± 0.5 | 2.1 ± 0.2 | | 0.84 ± 0.04 | 2.6 ± 0.2 | 1.8 ± 0.2 |
| | 40–55 | 6 | 20.8 ± 0.7 | 1.8 ± 0.3 | | 0.82 ± 0.03 | 2.1 ± 0.2 | 1.5 ± 0.2 |
| | 55–70 | 6 | 22.2 ± 0.8 | 1.5 ± 0.3 | | 0.82 ± 0.03 | 1.9 ± 0.2 | 1.2 ± 0.2 |
| | 70 | 6 | | | 23.4 ± 2.3 | | | |
| (μmole $ml^{-1}$) | (min) | | (μmole $ml^{-1}$) | (μmole $ml^{-1}$) | (μmole $g^{-1}$) | (ml $min^{-1}$ $g^{-1}$) | (μmole $min^{-1}g^{-1}$) | (μmole $min^{-1}g^{-1}$) |
| | 10–25 | 12 | 26.0 ± 0.3 | 2.1 ± 0.1 | | 0.98 ± 0.03 | 2.0 ± 0.1 | 2.0 ± 0.1 |
| | 25 | 6 | | | 4.2 ± 0.4 | | | |
| 33.3 | 25–40 | 6 | 27.3 ± 0.3 | 1.8 ± 0.2 | | 0.94 ± 0.04 | 2.0 ± 0.1 | 1.6 ± 0.1 |
| | 40–55 | 6 | 28.0 ± 0.3 | 1.7 ± 0.2 | | 0.90 ± 0.05 | 1.6 ± 0.1 | 1.5 ± 0.2 |
| | 55–70 | 6 | 28.5 ± 0.5 | 1.6 ± 0.2 | | 0.88 ± 0.04 | 1.6 ± 0.1 | 1.4 ± 0.2 |
| | 70 | 6 | | | 4.3 ± 0.3 | | | |

Perfusion velocity 0.1 ml $min^{-1}$, mean ± S.E.; concentration in intestinal wall, disappearance and appearance rate related to wet tissue weight.

concentration was accompanied by a 1000-fold increase in the appearance rate while the disappearance rate was increased only 700 to 800-fold and the accumulation in the wall only 180-fold.

The experiments show a discrepancy between the accumulation of L-phenylalanine in the intestinal wall and the appearance rate in the intestinal venous blood: the appearance rate increases in proportion to the luminal concentration up to 33.3 mM, while the accumulation in the intestinal wall already shows saturation kinetics. Since the disappearance rate includes the appearance and the accumulation rate, its deviation from proportionality is smaller. If the appearance rate depends on the concentration of the L-phenylalanine in the intestinal epithelial cell measured by the accumulation, a relatively smaller rate is to be expected at 33.3 mM. But the reported *in vivo* experiments show that the appearance rate does not parallel the accumulation in the intestinal wall. It is possible that the accumulation of L-phenylalanine is only a by-pass during the transfer of this substance from the intestinal lumen to the blood. The possibility of a different mechanism for accumulation and transfer of L-phenylalanine was previously discussed by Esposito et al. [4] based on their *in vitro* experiments. Furthermore, the finding that accumulation within the tissue is a saturable function whereas disappearance from the perfusate is not could explain the discrepancies between *in vitro* and *in vivo* findings, which are common in the intestinal literature.

The results reported here show the necessity to include the measurement of the appearance rate in the intestinal venous blood in an analysis of *in vivo* absorption mechanisms. The measurement of the disappearance rate from the intestinal lumen alone appears insufficient, since this rate includes the accumulation rate, the appearance rate in the intestinal blood and sometimes additionally the appearance rate on the serosal side [5].

**Acknowledgement**

The experiments were supported by the Deutsche Forschungsgemeinschaft.

## References

[1] D.M. Matthews and D.H. Smyth, J. Physiol. 126 (1954) 96.
[2] H. Newey and D.H. Smyth, J. Physiol. 145 (1959) 48.
[3] D. Winne and J. Remischovsky, Naunyn-Schmiedebergs Arch. Pharmak. 268 (1971) 392.
[4] G. Esposito, A. Faelli and V. Capraro, Arch. Sci. Biol. 48 (1964) 341.
[5] H. Ochsenfahrt, Naunyn-Schmiedebergs Arch. Pharmak. 270 Suppl. (1971) R 102.