

Leucine Absorption after Jejunoileal Bypass in Rats

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Jejunal and ileal absorption of L-leucine has been studied in rats subjected to jejunoileal bypass and in sham-operated rats, for five minute periods, using a perfusion technique. Aminoacid concentrations were: 1, 2.5, 5, 10 and 25 mM. In some experiments methionine was added to determine simple diffusion. The ratio of the active/diffusive components of absorption were calculated at the different luminal aminoacid concentrations in both groups of rats, showing that this ratio was lower in control animals.

Key words: Rat, Leucine, Intestinal absorption, Intestinal bypass, Leucine absorption.

The exclusion of segments of proximal and distal small bowel from normal continuity by surgical bypass provides the opportunity not only to study the effects of deprivation of luminal nutrition from the bypassed intestine, but also to examine the changes in the intestine that remains in continuity (9, 10). These changes are hypoplasia (in the bypassed segment), and hyperplasia (in the continuing intestine).

In the atrophic mucosa the non-electrolyte transport *in vivo* is diminished when expressed as absorption per cm of intestinal length (11), whilst in hyperplastic mucosa an increase in non-electrolytes transport *in vivo* is found when the same reference system is used (5, 13). On the other hand, experiments with isolated in-

testinal epithelial cells (21) or brush-border membrane vesicles (12) revealed that nutrient transport remains unaltered, indicating that the observed changes *in vivo* could be explained by alterations of the mucosal surface more than by alterations of the individual enterocytes.

The aim of the present work was to study, under *in vivo* conditions, the absorption of L-leucine in rat small intestine 3 months after jejunoileal bypass in both hyperplastic and hypoplastic intestinal segments, distinguishing between mediated and non-mediated aminoacid transport.

Materials and Methods

Bypass operation.— Male Wistar rats, 3 months old and weighing about 300 g,

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maintained on a standard pellet diet with free access to tap water, were used for these experiments. Animals, after a 24 h fast were anaesthetized with sodium pentobarbitone (4 mg/100 g b.w., i.p.). Laparotomy was then carried out and 45 cm jejunoileal bypass of the small bowel was performed starting 2 cm from the Treitz ligament, as previously described by NEMETH *et al* (15). Sham operations were performed on an equal number of rats, whose intestine was cut and re-anastomosed without bypass. In each instance, continuity of the gut was restored by end-to-end anastomosis (fig. 1). After 3 months, both groups of animals were used for absorption experiments.

In vivo absorption.—Rats were starved for 24 h and anaesthetized with sodium pentobarbitone (4 mg/100 g body wt, s.c.). Inflow and outflow cannulae were tied into jejunum and ileum; thus, jejunal and ileal loops about 20 cm were isolated from continuity with the lumen. The loops were rinsed with 0.9 % NaCl solution, replaced inside the body wall and perfused at a flow rate of 5.6 ml/min with prewarmed saline containing (mM): Na⁺, 150; Cl⁻, 135; HCO₃⁻, 15; K⁺, 5 and H₂PO₄⁻, 5 (pH = 7.4). Animals were maintained under controlled temperature. Multiple-pass perfusions of jejunal and ileal loops with saline solutions containing H³-labelled L-leucine or both L-leucine and methionine, over a range of concentrations, during a period of 5 min, were carried out. The absorption of the aminoacids was measured as luminal loss and was expressed in nmoles/cm² serosal surface/min.

Kinetic analysis.—The characterization of the intestinal absorption process rests upon the determination of the contribution of passive and non-passive components. The passive component was obtained in the presence of methionine, and

the K'_D (apparent mass-transfer-coefficient in nmol/cm²/min/mmol/l), was determined by linear regression analysis. The active transport kinetic constants were calculated from the curve obtained by fitting the data of non-passive transport with an unweighed single rectangular hyperbola (difference curve) by a developed program in an Apple II Europlus computer.

Tissue morphometric evaluation.—The outer circumference of the intestine was measured *in situ* during perfusion as has been described by WINNE (23). The villus surface and the mucosal surface area were calculated using the equation developed by ECKNAUER *et al* (8).

Statistics.—The results are presented as the average of the individual means with the standard error of the mean indicated. The significance of differences was determined by standard paired t test.

Materials.—L-(4,5-³H) Leucine was from Amersham International, and L-leucine and methionine were from Sigma. These and all other reagents were of A.R. grade.

Results

The body weight of sham-operated rats was significantly higher than that of bypassed animals, at three months from the surgical operation (Table I).

Intestinal tissue.—After 3 months of 45 cm jejunoileal bypass, the continuing ileum was hyperplased and the bypassed jejunum was hypoplasied (table I). Values for outer circumference and villus surface area showed statistical differences between sham and bypassed animals, in both jejunum and ileum. These values decreased in the bypassed jejunum and increased in the continuing ileum. The mucosal surface

Table I. *Effect of jejunoileal bypass on intestinal structural characteristics of the rat jejunum and ileum.*
Data are means $\pm$ S.E.M. P: In comparison with the respective sham-operated. Number of rats = 15.
n.s. = not significant.

Parameter	Sham-operated	Bypassed	P
<i>Body weight (g)</i>			
At start	358 $\pm$ 10	356 $\pm$ 11	n.s.
1 month after operation	418 $\pm$ 6	392 $\pm$ 14	n.s.
3 months after operation	463 $\pm$ 6	413 $\pm$ 12	0.001
<i>Jejunum</i>			
Outer circumference (cm)	2.10 $\pm$ 0.05	1.54 $\pm$ 0.07	0.005
Villus surface (mm ²)	0.578 $\pm$ 0.035	0.131 $\pm$ 0.008	0.001
Mucosal surface (mm ² /mm ² serosa)	7.9 $\pm$ 0.5	2.9 $\pm$ 0.2	0.001
<i>Ileum</i>			
Outer circumference (cm)	2.25 $\pm$ 0.08	2.61 $\pm$ 0.08	0.001
Villus surface (mm ²)	0.231 $\pm$ 0.011	0.437 $\pm$ 0.022	0.001
Mucosal surface (mm ² /mm ² serosa)	5.9 $\pm$ 0.3	6.6 $\pm$ 0.3	n.s.

area significantly decreased in the bypassed jejunum and slightly increased in the ileum.

Leucine absorption by sham and bypassed animals.— Jejunal aminoacid absorption by sham and bypassed rats, measured in the presence and absence of L-methionine at, 1, 2.5, 5, 10 and 25 mM

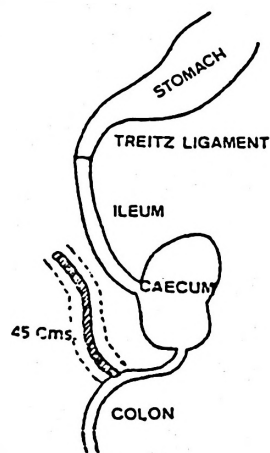


Fig. 1. *Intestinal bypass used in this study.* End-to-end with ileocolostomy. Hatching zone: bypassed segment.

concentrations in the bulk phase and related to serosal surface is shown in fig. 2. The relationship between total absorption and leucine concentration was non-linear in sham and bypassed jejunum (Curves A,

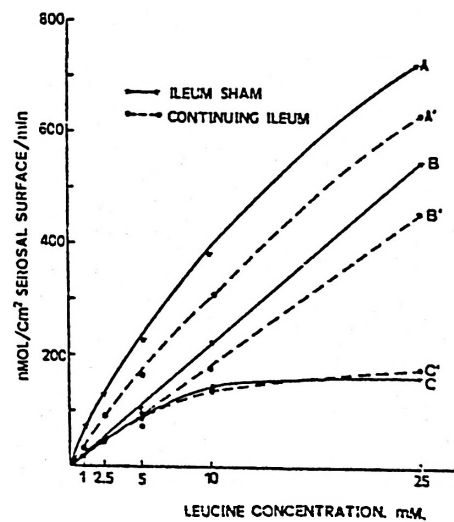


Fig. 2. *Jejunal leucine absorption by sham-operated and bypassed rats (dotted line).* Curve A and A', total absorption; curve B and B', passive component; curve C and C' non-passive component.

Table II. Effect of jejunoileal bypass on kinetic parameters of leucine absorption referred to serosal and mucosal surface.

Data represents means $\pm$ S.E.M. for fifteen animals. * $P < 0.001$. $K'_D = \text{nmol/cm}^2 \cdot \text{min} \cdot \text{mM}$. $K'_M = \text{mM}$. $J'_{\max} = \text{nmol/cm}^2 \cdot \text{min}$.

	Serosal			Mucosal		
	K'_D	K'_M	$J'_{\max}$	K'_D	K'_M	$J'_{\max}$
<i>Jejunum</i>						
Sham	25.9 ± 0.5	5.3 ± 0.1	146 ± 3	3.3 ± 0.1	5.2 ± 0.2	18 ± 0.5
Bypassed	$19.3 \pm 1.1^*$	$14.1 \pm 0.6^*$	$234 \pm 5^*$	$6.6 \pm 0.4^*$	13.9 ± 0.6	$80 \pm 6^*$
<i>Ileum</i>						
Sham	21.6 ± 0.7	6.3 ± 0.2	210 ± 15	3.8 ± 0.1	6.3 ± 0.2	35 ± 4
Continuing	$17.9 \pm 0.5^*$	$10.3 \pm 0.3^*$	248 ± 24	$2.7 \pm 0.1^*$	$10.2 \pm 0.3^*$	38 ± 5

A'). However, in the presence of 60 mM methionine a linear relationship was found in both animal groups (Curves B, B') revealing that this concentration was an effective inhibitor of L-leucine active transport. The slope of this line gives the K'_D , which is the apparent passive permeability coefficient for the non-mediated component. The results reveal that K'_D was smaller in bypassed animals (table II).

Mediated leucine transport was calculated by the difference between total absorption and non-mediated transport (absorption in the presence of methionine). When these values were plotted against the concentration of leucine in the bulk phase, saturation curves were obtained (Curves C, C'). In the sham jejunum the saturable component represents 46 % of the total absorption at 1 mM L-leucine and only 16 % at 25 mM L-leucine. This component increased slightly in bypassed jejunum (50 % and 23 % respectively). The apparent kinetics constants (K'_M and $J'_{\max}$), calculated by fitting the data with an unweighed single rectangular hyperbola, significantly increased in the bypassed jejunum after jejunoileal bypass (table II).

When these previous results were expressed with reference to mucosal surface, taking into account the data in Table I, new values of K'_D and $J'_{\max}$ were ob-

tained (table II): the K'_D in bypassed jejunum was higher than in sham jejunum and $J'_{\max}$ became about 5-fold higher in bypassed than in sham jejunum, instead of 1.7-fold found when the results were expressed as serosal surface.

Ileal aminoacid absorption by sham and bypassed animals was measured in the same experimental conditions as in jeju-

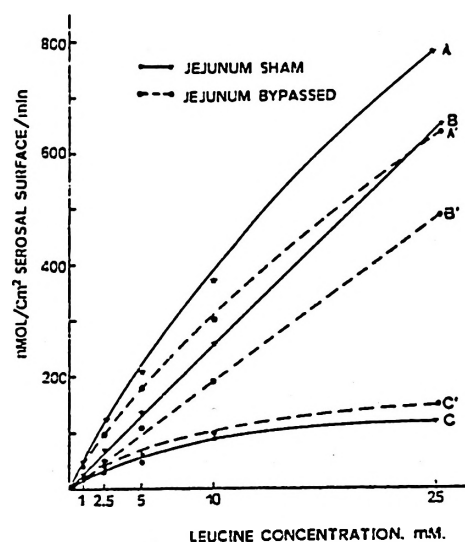


Fig. 3. Ileal leucine absorption by sham-operated and bypassed rats (dotted line).

Curve A and A', total absorption; curve B and B', passive component; curve C and C' non-passive component.

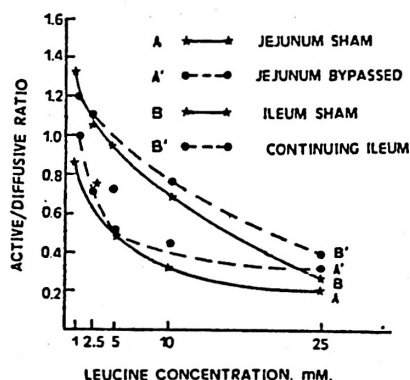


Fig. 4. Active/Diffusive ratio of L-leucine jejunal (A) and ileal (B) absorption in sham-operated and bypassed rats.

num. Results show a decrease in total ileal leucine absorption after jejunoileal bypass when referred to serosal or mucosal surface (fig. 3), but an increase when referred to intestinal length (data not shown). The passive component was a linear function of the substrate concentration, whose slopes have greater values in sham operated animals than those obtained in bypassed animals (table II).

In the range of leucine concentrations studied in the sham ileum, the non-passive component accounts for 56 % of the total absorption at 1 mM leucine, and only 23 % at 25 mM leucine concentration (fig. 3). This component was similar in the continuing ileum of the bypassed animals (52 % and 28 % respectively). After jejunoileal bypass, the continuing ileum showed an increase in K'_M and J'_{max} , similar levels remaining in sham and bypassed animals both expressed as serosal surface area and as mucosal surface area (table II).

The ratio of active (methionine-sensitive)/diffusive (methionine-insensitive) absorption indicates in both jejunum or ileum of sham or bypassed rats, that the active pathway is more important at low luminal aminoacid concentrations (fig. 4).

Discussion

There is growing evidence that deprivation of luminal nutrition, either by starvation or by surgical exclusion, leads to progressive structural atrophy (7, 17). Our findings are in accordance with the results reported by different authors after bypass (2, 14). The outer circumference (cm), villus surface (mm^2) and mucosal surface (mm^2/mm^2 serosa) decreased in bypassed jejunum as compared with sham jejunum (table I).

In the continuing ileum, opposite structural changes were found, showing that mucosa were hyperplastic, in accordance with others (6, 12, 16).

The absorption in bypassed jejunum 3 months after bypass, when both morphological and functional changes had reached the final stage, was studied. We found a decrease with respect to sham tissues in the K'_D values that are higher than those reported *in vitro* studies (4) and a significant increase in the kinetic parameters, verifying that the control K'_M values were quite similar to those determined *in vivo* (1, 3, 18, 19). In view of the fact that until now the individual enterocyte has been found to be unchanged in the atrophic mucosa (14, 20), our findings must be interpreted as indicative of an increased number of transport sites in the individual enterocytes or of a larger proportion of epithelial cells in the mucosa of bypassed loops (14).

Since, after jejunoileal bypass, a slight increase in mucosal surface has been shown (table I), the results of ileal leucine absorption, when referred to mucosal surface, revealed that K'_D decreased and K'_M increased after bypass. Regarding the differences in K'_D , these results should be interpreted by taking into account that in the atrophic (bypassed jejunum) or hypertrophic (continuing ileum) mucosa, changes in the unstirred water layer thickness can be expected. In accordance with the equation developed by WESTERGAARD

and DIETSCHY (22), the higher the unstirred water layer thickness, the greater the bias in the determination of the values of K'_D , so that the real differences in this parameter should be more attenuated than those shown for jejunum and ileum in Table III. The same reasoning can be applied to values of K'_M and J'_{max} . In this case a higher unstirred water layer thickness should produce a higher decrease in the K'_M and a higher increase in the J'_{max} values, in accordance with the theoretical model developed by WINNE (24). Thus, the real differences regarding K'_M values should be more attenuated in the ileum and more prominent in the jejunum, whereas J'_{max} values should be the opposite.

It is known that in the interdigestive periods, non-electrolites can leak into the lumen and its reuptake occurs by active transport. The bypassed jejunum, with its normal blood flow, can be considered to be permanently in the interdigestive period, so an increased affinity and capacity in the aminoacid active transport system could be more efficient.

On the other hand, the ratio active/difusive components slightly changed in jejunum and ileum of bypassed animals, revealing that for the leucine concentrations used, this ratio was lower in control animals.

Acknowledgment

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Resumen

Se estudia la absorción intestinal de L-leucina (1, 2,5, 5, 10 y 25 mM) en yeyuno e íleon de ratas sometidas a un bypass yeyunoileal y en patrones (ratas sham), utilizando técnicas de perfusión durante 5 min. Se utiliza en algunos experimentos la metionina, para determinar la difusión simple. La relación de los componentes activo/difusivo de la absorción se cal-

cula a diferentes concentraciones lumbales de aminoácido en ambos grupos de ratas, mostrando que esta relación es más baja en animales controles.

Palabras clave: Rata, Leucina, Absorción intestinal, Bypass intestinal, Absorción de leucina.

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