Interference between Leucine, Isoleucine and Valine during Intestinal Absorption

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1. The reciprocal interference between L-leucine, L-isoleucine and L-valine during absorption was studied in rats both *in vivo* and with an everted-sac preparation *in vitro*. 2. After feeding with the amino acids alone there was a considerable increase in their concentration in the intestinal lumen followed by a rapid disappearance, indicating efficient absorption. Absorption was reflected by a high concentration of the respective amino acids in the portal plasma. Isoleucine and valine inhibited the absorption of leucine, and leucine inhibited the absorption of isoleucine and valine. Inhibition of absorption by the interfering amino acid was generally partly overcome after 30–60 min., probably through the absorption of the interfering amino acid. At that time the rise in the concentration of the amino acid in portal plasma began. 3. These results were confirmed by experiments *in vitro*: isoleucine and valine inhibited the absorption rate of leucine, and leucine that of isoleucine and valine. 4. Active absorption of amino acids was rapid at low concentrations and depressed at higher concentrations.

A dietary excess of L-leucine depresses the growth of rats fed on a low-protein diet, but the impairment is largely overcome by the addition of valine or isoleucine (Harper, Benton, Winje & Elvehjem, 1954; Harper, Benton & Elvehjem, 1955; Benton, Harper, Spivey & Elvehjem, 1956; Spolter & Harper, 1963). It therefore appears that an excess of leucine inhibits the utilization of the structurally similar amino acids valine and isoleucine. Hagihira, Ogata, Takedatsu & Suda (1960), studying absorption from isolated sections of rat intestine, demonstrated reciprocal inhibition of absorption between valine and leucine or isoleucine. According to Kamin & Handler (1952) leucine inhibits the absorption of isoleucine by rat intestine, whereas the absorption of leucine is not inhibited by isoleucine. Spolter & Harper (1963) concluded that the depression of growth resulting from an excess of leucine is of metabolic origin rather than the result of an interference in some phase of digestion.

In the present study different aspects of leucine-isoleucine-valine interrelationships were investigated. In experiments *in vivo* we fed rats with each of these amino acids alone or with an excess of one of the other amino acids, and studied the correlations between the amounts of these amino acids administered and their concentrations in the intestinal lumen and portal plasma. With everted sacs, the absorption rate from the intestine and the concentration gradient of each amino acid were

investigated when the respective amino acid was present alone or when one of the other two amino acids was added.

METHODS

Experiments in situ. Seven-week-old rats of a local strain were used. During the week before the test they were fed on the following diet: casein, 18 parts; maize starch, 36 parts; glucose, 37 parts; vegetable oil, 5 parts; salt mixture (no. 2, U.S.P. XIII), 4 parts. This diet was supplemented with the conventional vitamins. At the end of the preparatory period the rats were starved for 48 hr. The relatively long starvation period was necessary for the complete removal of food residues from the intestine. The rats were then given a test meal containing the amino acid(s) studied with maize starch. The test meal consisted of 5 g. of starch suspended in 100 ml. of an aqueous solution of L-leucine and/or L-isoleucine and/or L-valine, 5 ml./100 g. body wt. being administered by stomach tube. The amino acid solutions contained 2.5, 5.0 or 7.5 m-moles of the respective amino acid/100 ml. Controls received a 5% starch suspension without amino acid. At 10, 30 and 60 min. after the test meal samples of portal plasma and intestinal contents were obtained as described by Goldberg & Guggenheim (1962). The samples were assayed for leucine, isoleucine and valine with *Lactobacillus arabinosus* 17/5 (*Lactobacillus plantarum* A.T.C.C.), as described by Barton-Wright (1952). The results indicated in the Figures are the averages of six to 12 observations. According to our experience the dose-response curve of the micro-organism to one of the amino acids of this group was not affected by an excess of another.

Table 1. *Effect of incubation time on the absorption rate and concentration gradient of leucine*

The results are given as means $\pm$ s.e.m., with the numbers of experiments in parentheses. The initial concentration of leucine was 1 mM. The absorption rate and concentration gradient are expressed as defined in the Methods section.

Incubation time	60 min.	120 min.	180 min.
Absorption rate	11 $\pm$ 0.11 (3)	18 $\pm$ 0.21 (4)	36 $\pm$ 3.30 (11)
Concentration gradient	1.69 $\pm$ 0.06 (3)	1.94 $\pm$ 0.08 (4)	2.56 $\pm$ 0.20 (11)

Experiments in vitro. Male rats weighing 130–150 g. were killed and a 15 cm.-long segment of the intestine just distal to the pylorus was removed and everted. The amino acids were dissolved in Krebs–Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932), containing 0.3% of glucose and placed within the sacs (1.0 ml.) at the same concentration as outside the sacs (25.0 ml.). The preparations were incubated in 50 ml. flasks that were continuously gassed with O₂ + CO₂ (95:5). The flasks were placed in a water bath (37°) with a moving rack. Absorption rates and concentration gradients of L-leucine, L-isoleucine and L-valine were measured when an amino acid was present alone or in the presence of different concentrations of the other amino acids. The absorption rate was expressed as $(C_s - C_m)/3 \text{ hr./mg. dry wt. of intestine}$ [C_s and C_m are concentrations (μM) of an amino acid in serosal and mucosal fluid respectively]. The concentration gradient is expressed as the ratio of final serosal to final mucosal concentration (Wiseman, 1964). The amino acids were assayed microbiologically (Barton-Wright, 1952). In preliminary experiments the effect of time of incubation on the absorption rate and concentration gradient of leucine was studied (Table 1). The highest values were obtained after 3 hr. An incubation time of 3 hr. was therefore adopted in all experiments. Initial and final volumes of the incubation fluids were determined and changes in volume were generally not observed. The results of the few experiments in which such changes occurred were rejected.

RESULTS

Experiments in situ. First, the concentrations of leucine in intestinal lumen and portal plasma were measured after the administration of starch or of a solution containing 2.5 m-moles of leucine/100 ml. (Fig. 1). When starch was given, leucine values in both intestine and portal plasma remained low and without great changes during the 60 min. observation period. The administration of leucine increased considerably the leucine content of the intestinal lumen after 10 min., but the amount decreased 20 min. later, indicating efficient absorption. The absorption was reflected by the concentration of leucine in portal plasma, which was already high 10 min. after its administration. The concentration gradually decreased during the next 50 min. The administration of solutions containing 5.0 or 7.5 m-moles of isoleucine or 7.5 m-moles of valine along with 2.5 m-moles of leucine/100 ml. resulted in a much higher leucine content of the intestinal

Fig. 1. Amount of leucine in the intestinal lumen (a) and concentration in the portal plasma (b) 10, 30 and 60 min. after the administration of starch (●) or of solution containing, per 100 ml., 2.5 m-moles of leucine alone (□) or with 5.0 m-moles of isoleucine (△), 7.5 m-moles of isoleucine (○) or 7.5 m-moles of valine (▲).

lumen after 10 min., indicating an inhibition of leucine absorption. The inhibition was still observed after 30 min. The leucine concentration in portal plasma generally mirrored that in the intestine. At 10 min. after the administration of leucine–isoleucine or leucine–valine mixtures, leucine concentrations in portal plasma were much lower than after feeding with leucine alone. At 30 and 60 min. after the test meal, however, they were considerably higher. At that time the inhibition of leucine absorption by isoleucine or valine was partly overcome as a result of the absorption of these amino acids, and the leucine concentration in portal plasma rose concomitantly with its final fall

Fig. 2. Amount of isoleucine in the intestinal lumen (a) and concentration in the portal plasma (b) 10, 30 and 60 min. after the administration of starch (●) or of solution containing, per 100 ml., 2.5 m-moles of isoleucine alone (□) or with 2.5 m-moles (▲), 5.0 m-moles (▽) or 7.5 m-moles (○) of leucine.

in the intestinal lumen. It appears to be characteristic of the kinetics of leucine concentration in portal plasma that the peak of the curve appeared 10 min. after the test meal when this amino acid was given alone, but at a later time when isoleucine or valine was also present.

The changes in the isoleucine contents of the intestinal lumen and portal plasma were similar (Fig. 2). When isoleucine was given alone, it disappeared rapidly from the intestinal lumen and its concentration rose in portal plasma. The simultaneous administration of leucine greatly decreased its disappearance rate from the intestinal lumen and resulted in a delayed rise in the concentration of this amino acid in portal plasma. With the largest dose of leucine, the isoleucine content of the intestinal lumen remained high and did not rise much in the portal plasma during the observation period. With isoleucine also maximal concentrations in portal plasma were found after the test meal when leucine was absent, whereas the presence of leucine caused a delay in the peak values.

Lastly, the effect of leucine on valine was studied (Fig. 3). When given alone, valine disappeared rapidly from the intestinal lumen but its disappearance was delayed when leucine was simultaneously administered. The higher the dose of

Fig. 3. Amount of valine in the intestinal lumen (a) and concentration in the portal plasma (b) 10, 30 and 60 min. after the administration of starch (●) or of solution containing, per 100 ml., 2.5 m-moles of valine alone (□) or with 2.5 m-moles (○), 5.0 m-moles (▽) or 7.5 m-moles (▲) of leucine.

leucine the higher the amount of valine remaining in the intestinal lumen during the first 30 min. Further, the valine concentration in portal plasma after 10 min. reflected its amount in the intestinal lumen. The administration of 5.0 or 7.5 m-moles of leucine/100 ml. resulted in a higher valine content after 30 min. than when valine was given alone or simultaneously with only 2.5 m-moles of leucine/100 ml.

Experiments in vitro. In these experiments the absorption rate and concentration gradient of each of the amino acids were studied when it was present alone or in the presence of an interfering amino acid (Table 2). Isoleucine significantly inhibits the absorption rate and decreases the concentration gradient of leucine. Its effect on the absorption rate increases with increasing concentration. Valine likewise inhibits the absorption rate of leucine. However, its effect on the concentration gradient of leucine is barely significant ($P=0.05$). Absorption rates as well as concentration gradients of isoleucine and valine are considerably depressed in the presence of leucine at a concentration of 2 mM. Leucine at a concentration of 1 mM, although

Table 2. *Absorption rates and concentration gradients of amino acids*

The results are given as means $\pm$ S.E.M., with the numbers of animals in parentheses. The initial concentration of the amino acids studied was 1 mM. The absorption rate and concentration gradient are expressed as indicated in the Methods section.

Amino acid	Parameter studied	Absorption rate and concentration gradient when amino acid present with						
		—	Isoleucine (1 mM)	Isoleucine (2 mM)	Isoleucine (3 mM)	Valine (3 mM)	Leucine (1 mM)	Leucine (2 mM)
Leucine	Absorption rate	36 $\pm$ 3.3 (10)	21 $\pm$ 3.5 (9)	20 $\pm$ 1.4 (12)	14 $\pm$ 0.8 (6)	16 $\pm$ 1.0 (5)	—	—
	Inhibition of absorption (%)	—	41	44	61	55	—	—
	Concentration gradient	2.56 $\pm$ 0.20 (10)	1.30 $\pm$ 0.16 (9)	1.74 $\pm$ 0.07 (12)	1.58 $\pm$ 0.04 (6)	1.84 $\pm$ 0.32 (5)	—	—
Isoleucine	Absorption rate	12 $\pm$ 2.0 (6)	—	—	—	—	8 $\pm$ 0.7 (9)	4 $\pm$ 0.8 (5)
	Inhibition of absorption (%)	—	—	—	—	—	33	66
	Concentration gradient	1.66 $\pm$ 0.13 (6)	—	—	—	—	1.44 $\pm$ 0.10 (9)	1.19 $\pm$ 0.05 (5)
Valine	Absorption rate	16 $\pm$ 1.1 (8)	—	—	—	—	10 $\pm$ 1.2 (5)	10 $\pm$ 1.4 (9)
	Inhibition of absorption (%)	—	—	—	—	—	37	37
	Concentration gradient	2.26 $\pm$ 0.26 (8)	—	—	—	—	1.28 $\pm$ 0.07 (5)	1.66 $\pm$ 0.15 (9)

Table 3. *Effect of the concentration of amino acids on their absorption rate and concentration gradient*

The results are given as means $\pm$ S.E.M., with the numbers of animals in parentheses. The absorption rate and concentration gradient are expressed as indicated in the Methods section.

Concn. (mM)	Leucine		Valine		Isoleucine	
	Absorption rate	Concentration gradient	Absorption rate	Concentration gradient	Absorption rate	Concentration gradient
1	36 $\pm$ 3.30 (10)	2.56 $\pm$ 0.20 (10)	16 $\pm$ 1.00 (8)	2.16 $\pm$ 0.44 (8)	12 $\pm$ 2.00 (6)	1.66 $\pm$ 0.13 (6)
2	29 $\pm$ 1.95 (5)	1.70 $\pm$ 0.15 (5)	21 $\pm$ 2.12 (5)	1.63 $\pm$ 0.09 (5)	8 $\pm$ 0.40 (8)	1.33 $\pm$ 0.04 (8)
3	14 $\pm$ 2.00 (5)	1.26 $\pm$ 0.10 (5)	7 $\pm$ 0.90 (5)	1.22 $\pm$ 0.07 (5)	—	—
5	17 $\pm$ 1.80 (9)	1.20 $\pm$ 0.09 (9)	0 (5)	0.94 $\pm$ 0.04 (5)	—	—
10	11 $\pm$ 1.50 (8)	1.07 $\pm$ 0.08 (8)	—	—	0 (5)	0.79 $\pm$ 0.01 (5)
15	0 (10)	0.90 $\pm$ 0.06 (10)	—	—	—	—

strongly inhibiting the absorption rates of isoleucine and valine and decreasing the concentration gradient of valine, is without any appreciable effect on the concentration gradient of isoleucine.

The results of these experiments confirm those of the experiments *in situ*.

During these experiments the effect of concentration on the active absorption of amino acids was studied (Table 3). The results indicate that the three amino acids studied are actively absorbed at low concentrations, but that active transport is progressively depressed when the concentrations are increased.

DISCUSSION

Our study indicates that L-leucine, L-isoleucine and L-valine interfere reciprocally during their absorption from the intestine. The interference of the absorption of leucine by isoleucine or valine and of the absorption of isoleucine and valine by leucine could be demonstrated both *in vivo* by the inhibition of disappearance of these amino acids from the intestinal lumen and the delayed rise of the concentration in portal plasma as well as *in vitro* by using an everted-sac preparation.

Whereas the existence of an antagonism between

these amino acids is well established, there is much uncertainty as to its explanation (Harper, 1964). One possible explanation is interference during absorption. Inhibition of the absorption of isoleucine by leucine (but not of that of leucine by isoleucine) has been demonstrated *in vivo* (Kamin & Handler, 1952), and reciprocal inhibition of absorption between valine and leucine and between leucine and isoleucine with sections of intestine (Hagihira *et al.* 1960). Rogers, Spolter & Harper (1962) fed rats on a high-leucine diet and found lower concentrations of isoleucine and valine in peripheral blood plasma than the respective concentrations in blood plasma from groups fed on the basal diet without leucine. In experiments on humans (Snyderman, Cusworth, Roitman & Holt, 1959) 25g. of leucine was administered to each of two adults. This resulted in a sharp rise in the concentration of this amino acid in blood plasma and a concomitant fall in that of valine. The omission of leucine from the diet of two infants decreased the plasma concentration of leucine and increased the concentration of valine.

It is possible that the interference of the absorption of amino acids impairs protein synthesis. This would partly explain the adverse effect of a dietary excess of individual amino acids. The digestion of proteins, the absorption of amino acids and their incorporation into body protein proceed very rapidly (Dawson & Porter, 1962). Protein synthesis in the body may therefore be impaired when the tissues are offered an amino acid mixture in which one amino acid is present in insufficient amount owing to an excess of another amino acid. Harper (1964), however, believes that 'the very great absorptive capacity of the small intestine argues against this being a major effect *in vivo*. Even though the absorption of isoleucine or valine may be delayed by an excess of leucine, as more leucine is absorbed, the ratio of leucine to isoleucine and valine will fall and competition for absorptive sites would diminish.' Although our results seem to support these suggestions, the possibility of an impairment of protein synthesis during the initial stages of amino acid absorption cannot be excluded.

Finally, our experiments demonstrate that leucine, isoleucine and valine are actively absorbed by the intestine at low concentrations but not at high concentrations. Similar effects of high con-

centrations have been reported for tryptophan (Cohen & Huang, 1964), phenylalanine (Spencer & Samy, 1961) and valine (Reiser & Christiansen, 1965). Cohen & Huang (1964) suggest that the depressing effect at higher concentrations might be due to inhibition by the compound of an enzyme system related to transport. If this interpretation is correct, it might also apply to the inhibition of absorption by related amino acids, as reported in Table 2. It would be caused by an unspecific overloading of a carrier system as well as by specific antagonism.

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