Effect of dietary changes on intestinal absorption of L-methionine and L-methionyl-L-methionine in the rat

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1. The absorption rates of L-methionine and L-methionyl-L-methionine (dimethionine) from the upper jejunum and lower ileum of the rat were studied in vivo after different dietary treatments. Rates were expressed per unit gut length and per unit gut weight; the former was considered to be the more satisfactory under the different dietary conditions.

2. The dietary treatments were either short-term (10 d) or long-term (40–84 d).

3. The rate of absorption of methionine increased in the jejunum after a restricted dietary intake, a high-protein diet or a high-methionine diet, but decreased after long-term protein deprivation. Short-term dietary restriction had a similar effect on methionine absorption in jejunum and ileum, though less pronounced in the latter. The rate of absorption of dimethionine was less influenced by dietary changes than that of methionine.

4. Under all conditions studied, the absorption rate of methionine was greater when presented as the dipeptide than when presented as the equivalent amount of free amino acid. This confirms that dimethionine is taken up intact from the intestinal lumen, and it seems likely that there are different mechanisms of mucosal uptake for methionine and its dipeptide.

Preliminary investigations of absorption rates of methionine and the dipeptide L-methionyl-L-methionine (dimethionine) after a short period of reduced dietary intake (a dietary condition referred to as 'semistarvation' by Crampton, Lis & Matthews, 1970) showed that reduced food intake increased the absorption of both amino acid and peptide, but that the effect on absorption of the peptide was less marked. This suggested that the mechanisms of intestinal uptake of the two compounds might not be identical. In view of this, and because we know of no other study of peptide absorption following several dietary alterations, we have investigated the effects of different diets on the rates of absorption of methionine and dimethionine.

Newey & Smyth (1959, 1960, 1962) first showed that mucosal uptake of dipeptides, with cellular hydrolysis, occurred in the small intestine of the rat and dog. Studies of relative absorption rates of amino acids and small peptides and investigations of defects in intestinal amino acid transport have now shown that mucosal uptake of small peptides occurs in many mammalian species, including man (Matthews, Craft, Geddes, Wise & Hyde, 1968; Craft, Geddes, Hyde, Wise & Matthews, 1968; Tarlow, Seakins, Lloyd, Matthews, Cheng & Thomas, 1970; Hellier, Perrett & Holdsworth, 1970; Gangolli, Simson, Lis, Crampton & Matthews, 1970; Lis, Crampton & Matthews,

1971). The relationship between amino acid and peptide uptake mechanisms is not yet clear, but recent observations on the amino acid transport defect of Hartnup disease (Asatoor, Cheng, Edwards, Lant, Matthews, Milne, Navab & Richards, 1970) indicate that they may be partly or completely independent, as they are in bacteria (Payne, 1968). The results of the present experiments support this concept.

L-Methionine and L-methionyl-L-methionine were chosen for the investigation because it has previously been shown that absorption of methionine units is considerably more rapid from the dipeptide than from the equivalent amount of amino acid, which shows that this peptide must be taken up intact by the intestinal mucosa (Matthews, Lis, Cheng & Crampton, 1969; Cheng, Navab, Lis, Miller & Matthews, 1971).

MATERIALS AND METHODS

Male rats of the Sprague–Dawley (Specific Pathogen Free) strain were used, weighing 200–300 g initially. Animals were housed in individual cages and food was presented in small pots, allowing daily determination of food consumption. Water was allowed *ad lib.*

Dietary treatments

The calculated compositions of the various diets are shown in Table 1, together with the composition of the vitamins and salts added. All diets were given *ad lib.*, except that in a separate treatment the standard diet was given in restricted amounts of about 50% of the corresponding *ad lib.* intake at which level body-weights remained approximately constant. Short-term (10 d) or long-term (40–84 d) experiments were carried out.

Table 1. *The percentage composition of the different diets and their contents of total protein, fat and carbohydrate*

	Standard diet	'5% protein'	High-protein	High-methionine
Cube diet (Spillers Ltd)	100	25	19	96·15
'Protein-free diet'*	—	75	—	—
Casein (Fisons Ltd)	—	—	76	—
Maize oil	—	—	5·0	—
L-Methionine	—	—	—	3·85
Calculated composition (%):				
Crude protein (N × 6·25)	21·0	5·6	68·6	20·2
Ether extractives	5·1	4·6	6·0	4·9
Carbohydrate (starch + sugar)	51·0	74·6	9·7	49·0

* Prepared by mixing 50 ml maize oil with 1 kg maize starch, fortified with the following vitamins: vitamin A 5000 i.u.; ergocalciferol 300 i.u.; vitamin E (α-tocopherol) 60 mg; thiamin 4 mg; riboflavin 5 mg; vitamin B_6 6 mg; nicotinic acid 10 mg; calcium pantothenate 12 mg; vitamin B_{12} 5 μg; choline chloride 1·0 g; vitamin K (menaphthone) 1·5 mg; and the following minerals (mg): KH_2PO_4 3200; $CaCO_3$ 3200; NaCl 700; $MgSO_4$ 288; $FeSO_4.7H_2O$ 144; $MnSO_4.2H_2O$ 21·6; KI 3·5; $CuSO_4.5H_2O$ 1·2.

Experimental methods

Absorption was estimated by disappearance of methionine or dimethionine from tied loops of upper jejunum. Experimental technique and analytical methods were as described by Matthews *et al.* (1968). Either L-methionine (200 mmol/l) or L-methionyl-

L-methionine (dimethionine) (100 mmol/l) was introduced into adjacent jejunal loops. The solutions were made up to 300 mmol/l with mannitol. In studies of ileal absorption, the technique was similar but loops were prepared from the ileum between 10 and 30 cm from the ileo-caecal valve.

The length of the small intestine was measured, after stripping off the mesentery, by holding the gut vertically against a ruler. The wet weight was taken after gently squeezing out the gut contents and removing fragments of mesentery and adherent fat. The wet weight of loops used for measurement of absorption was obtained after draining and blotting at the end of the absorption experiment and the dry weight after overnight drying in an oven at 110°.

Absorption was measured in μmol methionine units disappearing from the lumen in 10 min, and expressed on a length basis (per cm of small intestine) or on the basis of wet or dry weight of intestine.

In general, results for animals on experimental diets were compared with results for animals of similar age fed on the standard diet. Differences between means were taken to be significant when $P < 0.05$ (t test).

RESULTS

Changes in body-weight

Rats on the 'protein-free' diet lost weight and their food intake was reduced, as in the animals on the high-methionine diet. Those on the 5% protein diet failed to gain weight during the short-term feeding experiments, as did the animals on reduced intake of the standard diet. There was initially a slight reduction in food intake and loss in weight of animals on the high-protein diet. Prolonged feeding of the 5% protein diet reduced the growth rate.

Changes in gut weight and length

Table 2 shows changes in total weight and length of the small intestine after short-term and long-term feeding on different diets. The older group of control animals had a slightly longer and lighter gut than the younger group. There were no marked changes in gut length after different diets, except for a slight increase on the high-protein diet and a slight decrease after a prolonged period on the 5% protein diet. Reductions in total gut weight were very pronounced after food restriction, the 'protein-free' diet and high-methionine diet. The final gut weight and body-weight of rats on the protein-free diet were less than those of rats on reduced intake of standard diet, although the total calorie intake was similar in both groups of animals. After 10 d the gut weight of animals on the 5% protein diet was lower than that of the controls, but after prolonged feeding it was no lower.

Jejunal absorption rates

Absorption rates of methionine from the dipeptide were greater than from the equivalent amount of free amino acid after all the diets studied (Table 3).

When absorption rates were expressed on the basis of wet or dry weight,

Table 2. *Change in body-weight, gut weight and length, and food intake of rats on different diets*

Dietary conditions and duration	Body-weight, final* (g)	Body-weight change (g)	Total gut length (cm)*	Total gut wet weight* (g)	Food intake† (g/d)
10 d expt:					
Standard diet (controls)	300 ± 10·4 (12)	+50	102·7 ± 2·43	5·65 ± 0·12	25 (20–30)
Restricted intake	265 ± 5·8 (8)	−7	103·0 ± 1·32	4·30 ± 0·22	10 (10–12)
'Protein-free'	187 ± 3·1 (8)	−57	104·0 ± 2·12	3·90 ± 0·11	14·5 (10–30)
5% Protein	222 ± 4·6 (4)	+1	101·0 ± 0·96	4·40 ± 0·16	22 (17–30)
High-protein	304 ± 2·6 (8)	+22	114·6 ± 2·35	5·90 ± 0·13	24 (17–30)
High-methionine	228 ± 3·4 (8)	−9	106·8 ± 2·17	4·25 ± 0·13	20 (6–30)
Long-term expt:					
Standard diet (controls)	460 ± 9·5 (10)	+215	114·3 ± 1·01	5·00 ± 0·22	25 (20–30)
Restricted intake (51 d)	301 ± 6·5 (8)	+8	108·8 ± 1·23	3·5‡ ± 0·18	13 (12–17)
'Protein-free' (41 d)	213 ± 4·4 (4)	−76	105·0 ± 7·13	2·80 ± 0·13	12 (4–24)
5% Protein (42 d)	332 ± 4·1 (8)	+55	108·0 ± 2·09	5·50 ± 0·13	24 (17–30)
5% Protein (84 d)	393 ± 17·3 (8)	+111	105·5 ± 1·79	4·60 ± 0·19	24 (17–30)

* Mean values with their standard errors; values in parentheses are the nos. of observations.
† Mean over whole period under investigation; ranges in parentheses.
‡ Mean of only three observations.

dimethionine was absorbed faster in the older group of control animals than in the younger group. As marked changes in intestinal weight occurred after different diets, absorption rates expressed on the basis of weight are considered separately from those expressed on the basis of length.

Absorption rates per unit length of jejunum. Absorption rates of methionine and dimethionine in animals after all the diets studied were compared with those of the control group for the corresponding period (Table 3). Significant differences between groups are summarized in Table 4.

Short-term reduction of food intake, and also the high-methionine and high-protein diets, increased the absorption rate of methionine. Dimethionine absorption was not significantly affected. Short-term feeding on the 'protein-free' and 5% protein diet did not alter the rate of absorption of methionine; that of dimethionine decreased.

Long-term reduction of food intake had no effect on the absorption rate of methionine; that of dimethionine was slightly increased. There was a decrease in the absorption rate of methionine after prolonged feeding on the 'protein-free' diet; dimethionine absorption was unaffected. Long-term feeding on the 5% protein diet did not alter the absorption rate of methionine or dimethionine.

Absorption rates per unit wet or dry weight of jejunum. Short-term restriction of food intake and feeding on the high-methionine diet increased absorption rates. There was an increase in absorption on the basis of dry weight after short-term feeding on the 'protein-free' and high-protein diet; long-term feeding on the 'protein-free' diet did not alter absorption rates on this basis. Prolonged food restriction increased the absorption rates of both methionine and dimethionine. Feeding on the 5% protein diet did not alter absorption rates.

Table 3. *Effects of different diets on absorption rates of methionine and dimethionine expressed on the basis of unit length and unit weight of jejunum of rats*

Dietary conditions and duration	Absorption rates*: μmol methionine/cm		μmol methionine/g wet weight		μmol methionine/g dry weight		Loop weight†	
	Methionine	Dimethionine	Methionine	Dimethionine	Methionine	Dimethionine	Wet weight (g/cm)	Dry weight (g/cm)
Short-term feeding (10 d):								
Standard diet	2·8 ±0·16 (14)	5·4 ±0·26 (13)	38·1 ±2·08	70·9 ±3·07	132·3 ±7·14	254·0 ±11·75	0·075	0·022
Restricted intake	4·2 ±0·34 (8)	6·0 ±0·25 (8)	78·0 ±4·71	110·0 ±3·22	350·0 ±20·21	500·0 ±22·03	0·054	0·012
'Protein-free'	2·4 ±0·11 (7)	4·3 ±0·21 (7)	45·0 ±2·96	84·9 ±2·82	170 ±12·02	332 ±14·04	0·052	0·014
5% Protein	2·5 ±0·25 (6)	4·2 ±0·19 (6)	37·5 ±2·34	74·0 ±6·34	113 ±11·01	257 ±17·34	0·062	0·018
High-protein	3·5 ±0·24 (8)	5·7 ±0·34 (8)	49·5 ±3·01	78·2 ±3·07	208 ±11·44	330·5 ±15·04	0·071	0·017
High-methionine	3·5 ±0·19 (8)	5·9 ±0·27 (8)	59·6 ±2·84	100·8 ±3·04	268 ±12·95	452 ±14·87	0·057	0·013
Long-term feeding:								
Standard diet	2·3 ±0·13 (11)	4·6 ±0·25 (8)	41·5 ±3·34	82·6 ±2·89	155·5 ±11·89	315·3 ±12·10	0·054	0·015
Restricted intake (51 d)	2·8 ±0·23 (9)	5·5 ±0·33 (6)	58·3 ±4·83	116·3 ±10·12	223 ±15·67	463·5 ±42·25	0·050	0·013
'Protein-free' (41 d)	1·2 ±0·38 (5)	4·1 ±0·38 (5)	30·7 ±9·28	99·8 ±6·44	104 ±30·99	349 ±31·74	0·040	0·012
5% Protein (42 d)	2·5 ±0·24 (8)	5·2 ±0·42 (8)	37·0 ±3·07	78·4 ±4·33	136 ±14·05	291 ±18·46	0·067	0·018
5% Protein (84 d)	2·1 ±0·19 (8)	4·3 ±0·27 (8)	42·2 ±3·86	84·7 ±6·52	137 ±12·54	280 ±22·20	0·051	0·016

* Mean values with their standard errors; values in parentheses are the nos. of observations.
† Each value is the mean of ten to nineteen determinations and has a standard error of 0·002 or less.

Ileal absorption rates

The effect of short-term reduction of food intake on ileal absorption rates is shown in Table 5. There was no significant difference on the basis of intestinal length, although the apparent increase in methionine absorption approached significance ($0{\cdot}1 > P > 0{\cdot}05$). There was an increase in absorption rates when expressed on the basis of wet or dry weight.

DISCUSSION

The results show that in a study of this kind, intended to determine the effects of various dietary alterations on the absorptive ability of the small intestine, it is more satisfactory to express absorption rates on the basis of gut length than on the basis of wet or dry weight. The length of the small intestine is little altered by the different diets, whereas in several instances (long-term food restriction and short- or long-term 'protein-free' diet) there is a substantial reduction in its weight. This can give rise

Table 4. *Statistically significant differences in jejunal absorption rates between rats on experimental diets and control rats*

Group	Absorption rates: μmol/cm		μmol/g wet weight		μmol/g dry weight	
	Methionine	Dimethionine	Methionine	Dimethionine	Methionine	Dimethionine
Short-term expt:						
Restricted intake	↑	NS	↑	↑	↑	↑
'Protein-free'	NS	↓	NS	↑	↑	↑
5% protein	NS	↓	NS	NS	NS	NS
High-protein	↑	NS	↑	NS	↑	↑
High-methionine	↑	NS	↑	↑	↑	↑
Long-term expt:						
Restricted intake (51 d)	NS	↑	↑	↑	↑	↑
'Protein-free' (41 d)	↓	NS	NS	↑	NS	NS
5% protein (42 d)	NS	NS	NS	NS	NS	NS
5% protein (84 d)	NS	NS	NS	NS	NS	NS

↑ = significantly increased. ↓ = significantly decreased. NS = no significant change.

Table 5. *Effect of short-term food restriction on ileal absorption of methionine and dimethionine, expressed on the basis of unit length and unit weight*

(Mean values with their standard errors; values in parentheses are the nos. of observations)

Dietary condition	Absorption rates: μmol/cm		μmol/g wet weight		μmol/g dry weight	
	Methionine	Dimethionine	Methionine	Dimethionine	Methionine	Dimethionine
Standard diet	1·3 ±0·39 (4)	2·5 ±0·38 (4)	17·7 ±4·31	37·7 ±5·70	70·9 ±18·09	156·3 ±22·14
Restricted intake	2·1 ±0·24 (5)	3·4 ±0·29 (6)	43·4 ±5·57	69·9 ±6·17	181·2 ±27·04	285·0 ±30·23

to misleading results when the weight basis is used for expressing absorption. For example, if it is found that a particular condition increases absorptive ability per cm in one part of the small intestine, and the total length of the gut is unchanged, then it is clear that if a similar change (or no change) occurs in other parts of the small intestine, total absorptive ability must be increased. If, on the other hand, there is no change in absorptive ability per cm length at any site in the gut, then total absorptive ability is unchanged. Yet should there be a concurrent fall in the weight of the gut, there will be an apparent increase in absorption on the weight basis. Such an increase may mean that the absorptive ability of each mucosal cell is increased – but it does not mean an increase in total absorptive ability. The use of different bases for expression of absorption can give conflicting results. This is illustrated by the effects of short-term feeding on the 'protein-free' diet. On the basis of length, absorption is decreased, but on the basis of wet or dry weight it is increased.

The conclusion that the amount absorbed per unit weight is not a valid measure of

total absorptive capacity agrees with that of Newey, Sanford & Smyth (1970) and of Craft (1970). The question of the most satisfactory basis for expression of absorption rate is difficult (Levin, Newey & Smyth, 1965; Levin, 1967; Newey & Smyth, 1967) and the use of a wide variety of different bases in experiments in vivo and in vitro (Kujalova & Fábry, 1960; Ziemlański, Cieślak, Pliszka & Szczygiel, 1967; Esposito, 1967; Kirsch, Saunders & Brock, 1968; Steiner & Gray, 1969; Adibi & Allen, 1970) makes comparison of results obtained by different investigators on the effects of dietary changes on absorption almost impossible. The problem is further complicated by the use of widely different concentrations of the substance under investigation (Steiner & Gray, 1969; Steiner, Farrish & Gray, 1969; Debnam & Levin, 1970), the fact that experiments in vivo have been carried out with and without added glucose (Kershaw, Neame & Wiseman, 1960; Wright & Barber, 1969), which affects amino acid transport (Newey *et al.* 1970), and the existence of species differences (Hindmarsh, Kilby, Ross & Wiseman, 1967).

The present results show that short-term reduction of food intake increased methionine absorption in the jejunum, regardless of the basis of expression. A similar trend occurred in the ileum, though on the basis of length it did not quite reach statistical significance. The results suggest that the absorptive ability of the small intestine as a whole was increased. The same treatment did not cause a significant increase in the absorption of dimethionine in the present experiments though we found previously that it caused a slight increase in jejunal absorption of dimethionine at a higher concentration (Crampton *et al.* 1970). The results with methionine are in general agreement with previous observations on amino acid absorption when food intake is reduced (Neame & Wiseman, 1959; Kershaw *et al.* 1960; Hindmarsh *et al.* 1967; Neale & Wiseman, 1969; Madge, 1970). Newey *et al.* (1970) observed that complete deprivation of food for 3 d caused an increase in amino acid transport by rat gut in vitro in the absence of glucose but a decrease in the presence of glucose.

Wiseman, Neame & Ghadially (1959) reported that reduction of food intake for 9 d caused thinning of all layers of the small intestinal wall, with reduction in size and number of villi and mucosal epithelial cells, and complete starvation also results in a reduction in the size and number of the mucosal cells (Hopper, Wannemacher & McGovern, 1968; Steiner *et al.* 1969). However, we agree with the conclusion of Wiseman *et al.* (1959) that the increase in amino acid absorption associated with short-term reduction of food intake is not due simply to thinning of the intestinal wall, because (1) the effect was transient, disappearing in 51 d, (2) the 'protein-free' diet caused a much greater loss in gut weight than short-term food reduction without increasing absorption, and (3) there was no significant increase in jejunal absorption of dimethionine. We also agree that the phenomenon is likely to represent a functional response. The mechanism of this response is not yet explained.

The decrease in jejunal absorption of methionine produced by the long-term 'protein-free' diet might be the result of reduction in mucosal area (Hopper *et al.* 1968) or decreased synthesis of carrier protein, or both. The latter explanation could account for the fact that in short-term protein deprivation the food intake falls, so that the animals are also underfed, but the response to simple food reduction – an increase in

methionine transport – is abolished. The fact that short-term protein deprivation reduces dimethionine absorption per cm jejunum, but not methionine absorption, is unlikely to be accounted for by the fall in mucosal peptidase activity reported by Solimano, Burgess & Levin (1967). This fall was expressed on a weight basis, and our results show that, when dimethionine absorption is expressed on a similar basis, it is not reduced but is increased.

The increase in jejunal absorption of methionine, but not of dimethionine, resulting from short-term feeding on the high-casein and high-methionine diets might be attributed to substrate stimulation of the methionine uptake systems but, since both these diets led to a reduction in food intake, the response may have been to reduced food intake rather than to the primary alteration in the diet.

A prominent feature of the study is that, in general, jejunal absorption of methionine was altered to a much greater extent by different diets than that of dimethionine. Table 3 shows that, expressed per cm jejunum, methionine absorption rates varied by a factor of more than three according to different conditions, whereas the highest rate of absorption of dimethionine was less than 50% greater than the lowest.

Though the possibility that this dissociation of effects is due to alterations in mucosal peptidases has not been excluded, it seems unlikely that such alterations could be entirely responsible, and we take the phenomenon to support the hypothesis that mucosal uptake of amino acids and dipeptides involves separate mechanisms.

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