# Absorption of L-Methionine from the Human Small Intestine

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A B S T R A C T   Absorption of L-methionine was
measured in all parts of the human small intestine
using transintestinal intubation and perfusion. In
four normal subjects, adsorption was higher in
the proximal than in the distal intestine. In two
patients with nontropical sprue in relapse, there
was a proximal zone of low absorption with higher
absorption distally. In all parts of the small intes-
tine, absorption showed rate-limiting kinetics as
methionine concentration was increased. In nor-
mal subjects, the proximal $K_m$ (Michaelis con-
stant) was more than 3 times higher than the
distal, which suggests a difference in transport
mechanisms between the two segments.

## INTRODUCTION

Several specific mechanisms have been implicated
in the uptake of amino acids by cells (1–3) as
well as by the intestinal mucosa (4, 5). Despite
extensive studies of amino acid transport in a
variety of preparations, information on amino acid
absorption by the human small intestine is limited
(6–10). We studied the pattern of L-methionine
absorption in the human small intestine and found
differences in transport characteristics between
the proximal and distal segments.

## METHODS

*Subjects.* Two normal men (M. R., age 44, and R. L.,
age 37) were volunteers, and the third (H. E., age 62)
was an ambulatory patient with generalized atherosclero-
sis. The woman control (B. J., age 44) was a volunteer.
The two patients with adult celiac disease were a 62 yr
old man, G. H., and a 60 yr old woman, G. L. The diag-
nosis was made on the basis of the clinical picture, ab-
normal routine absorption tests, the characteristic
changes in the small intestinal mucosa, and the response
to a gluten-free diet. The disease was in relapse in both
patients, and both were eating gluten-free diets during
the period of study. All subjects were hospitalized.

*Technique.* Absorption was studied by transintestinal
intubation (11) using a polyvinyl tubing (Pharmaseal
Laboratories, Glendale, Calif.) and a steady-state per-
fusion technique (12, 13). Subjects fasted overnight and
were studied in the recumbent position at the same time
each morning. Solutions were pumped into the nasal end
of the tubing at 15 ml/min using a constant infusion
pump (Bowman pump, Process & Instruments Co.,
Brooklyn, N. Y.) The solutions entered the gut through
a hole in the side of the tubing above a solid connector.
Perfusate was sampled through a similar hole, 15 cm
distal to the entry site and below the solid connector, by
siphonage from the anal end of the tube. Perfusion sites
were in the small intestine and were designated by dis-
tance in centimeters of the proximal hole from the nares.
Three solutions, containing different concentrations of
L-methionine, were perfused during each run. Usually the
solution with the lowest concentration was perfused first,
and the one with the highest last. The order of perfusion
was reversed without apparent effect on absorption rate
in about one-fifth of the runs. After an initial 30 min
wash, three to four successive samples were collected
over 4–10-min periods, depending on sample collection
rate. Sample volume (usually more than 20 ml), collec-
tion time, and pH were measured. A 30–40 min wash pe-

TABLE I

*L-Methionine Absorption in Relation to Concentration*

| Subjects mmoles/liter...............3 | Concentration perfused | | |
| --- | --- | --- | --- |
| | 3 | 6 | 12 |
| | *mmoles/hr per segment, mean ± SD* | | |
| | | Proximal | |
| **Normal** | | | |
| R. L. | | 3.6 ± 0.7 (3.6 ± 0.8)*(27)‡ | 8.1 ± 0.9 (6.5 ± 0.9) (26) |
| M. R. | | | |
| B. J. | | 4.5 ± 0.3 (3.0 ± 0.3) (5) | 7.4 ± 1.0 ( 7.3 ± 0.9) (9) |
| H. E. | | | 6.5 ± 1.0 (7.9 ± 0.7) (38) |
| **Sprue** | | | |
| G. H. | 1.5 (2.0) (2) | 1.6 ± 0.5 (4.8 ± 0.4) (8) | 3.2 ± 0.8 (9.3 ± 0.4) (7) |
| G. L.§ | | 2.8 (4.2) (2) | 3.8 ± 0.9 (9.4 ± 0.3) (6) |
| | | Distal | |
| **Normal** | | | |
| R. L. | 1.6 ± 0.3 (2.0 ± 0.3) (20) | 2.8 ± 0.4 (4.3 ± 0.3) (19) | 3.9 ± 0.8 (10.0 ± 0.5) (26) |
| M. R. | | | |
| B. J. | 2.2 (1.5) (3) | 3.3 ± 0.4 (4.0 ± 0.3) (15) | 4.8 ± 0.8 (9.3 ± 0.5) (24) |
| H. E. | | 4.0 ± 0.3 (3.2 ± 0.3) (11) | 5.2 ± 1.4 (8.9 ± 0.9) (11) |
| **Sprue** | | | |
| G. H. | | 4.2 ± 0.5 (2.9 ± 0.3) (7) | 6.1 ± 1.9 (7.9 ± 1.1) (8) |
| G. L. | | | 5.2 ± 0.6 (8.4 ± 0.5) (6) |

* Geometric mean concentration.

‡ Number of samples.

§ Proximal samples are from low rate proximal zone, distal are from the high rate zone (Fig. 2).

riod preceded sample collection from each succeeding solution.

*Solutions.* L-methionine (N.R.C., A Grade, Calbiochem, Los Angeles, Calif.) solutions in a modified Ringer's [pH = 7.0 ± 0.2 (14)] were maintained isotonic by reducing sodium chloride concentration 0.5 mmole/liter for each mmole of L-methionine per liter. All solutions contained polyethylene glycol (PEG) (Carbowax 4000, Carbide and Carbon Chemicals Co., New York) at a concentration of 1 mg/ml. Phenol red (5–25 mg/liter, depending on methionine concentration) was added to the second solution to check completeness of wash, i.e., absence of first solution from second, and second solution from third.

*Analysis.* Methionine was analyzed by a modification of the method of Horn et al. (15). The standard curve, read at 520 m$\mu$ in the Beckman DU spectrophotometer, was linear to above 2.4 mg of methionine. Sample aliquots approximating 2 mg of methionine in the perfusion solution were analyzed in duplicate. Sample banks containing all components except nitroprusside were run with the samples. Application of the three wave length corrections (16) to readings of samples and standards at 480, 520, and 560 m$\mu$ gave essentially the same results as reading at 520 m$\mu$ only. No methionine was found on analysis of perfusates from solutions lacking methionine.

PEG was analyzed turbidimetrically by a modification

of the method of Hydén (17). The ratio of initial to final PEG concentrations (PEG ratio) was used to correct for net water movement. L-methionine absorption was calculated as follows:

(*a*) Per cent unabsorbed =

$$100 \frac{\text{(final L-methionine concn)}}{\text{(initial L-methionine concn)}} \times \text{(PEG ratio)} ;$$

(*b*) Percent absorbed = 100 − Per cent unabsorbed;

(*c*) Absorption, mmoles/hr per gut segment =

$$\frac{\text{(Per cent absorbed)}}{100} \times \text{(Initial L-methionine concn,}$$

mmoles/liter) × (Pump rate, liters/hr) ;

(*d*) Geometric mean concn =

$$\sqrt{\text{(initial concn) (final concn)}}.$$

Data were plotted as described by Lineweaver and Burk (18), and $K_m$ and $V_{max}$ were obtained from the slopes and intercepts of lines fitted by the method of least squares.

## RESULTS

The pattern of small intestinal methionine absorption is shown in Fig. 1 for normal subjects and in Fig. 2 for patients with nontropical sprue. These absorption data are combined to compare proximal

TABLE I—(*Continued*)

*in Proximal and Distal Small Intestine*

| | Concentration perfused | |
|---|---|---|
| 25 | 50 | 100 |

*mmoles/hr per segment, mean ± SD*

Proximal

| 25 | 50 | 100 |
|---|---|---|
| 11.2 ± 2.2 (19.0 ± 1.6) (31) | 20.2 ± 3.7 (40.0 ± 2.8) (15) | 21.4 ± 5.7 (86.9 ± 3.6) (5) |
| 13.0 ± 1.4 (17.7 ± 1.2) (11) | 16.5 ± 3.1 (42.6 ± 1.2) (8) | 27.2 ± 5.4 (85.7 ± 6.2) (12) |
| 14.3 ± 3.0 (16.6 ± 2.2) (12) | 21.0 ± 5.9 (40.0 ± 2.7) (7) | |
| 9.4 ± 2.2 (19.9 ± 1.8) (41) | 13.2 ± 2.9 (44.4 ± 1.9) (25) | |
| | | |
| 4.6 ± 2.1 (20.7 ± 1.3) (12) | 8.3 ± 2.1 (41.7 ± 2.7) (9) | 6.4 ± 2.0 (92.6 ± 3.8) (9) |
| 5.0 ± 0.7 (21.8 ± 0.3) (6) | 6.0 ± 3.3 (42.7 ± 3.9) (12) | 8.1 ± 5.0 (89.8 ± 5.1) (4) |

Distal

| 25 | 50 | 100 |
|---|---|---|
| 4.1 ± 0.9 (23.5 ± 0.6) (40) | 7.8 ± 2.8 (47.2 ± 1.7) (31) | 9.4 ± 3.3 (94.7 ± 1.9) (18) |
| 5.3 ± 0.8 (22.4 ± 0.6) (12) | 8.0 ± 1.7 (47.3 ± 1.0) (15) | 11.3 ± 3.6 (95.9 ± 2.0) (13) |
| 5.8 ± 1.3 (22.1 ± 1.1) (28) | 10.0 ± 4.0 (45.9 ± 3.2) (13) | |
| 6.0 ± 1.4 (21.7 ± 0.9) (15) | 6.2 ± 2.8 (47.2 ± 0.9) (14) | |
| | | |
| 15.2 ± 1.2 (16.7 ± 1.1) (5) | 23.0 ± 4.5 (42.1 ± 4.0) (8) | 24.7 ± 13.9 (87.9 ± 5.8) (7) |
| 10.1 ± 4.2 (19.1 ± 3.0) (9) | 16.8 ± 6.7 (43.8 ± 5.2) (28) | 21.5 ± 7.1 (85.0 ± 8.5) (19) |

and distal rates in relation to geometric mean concentration in Table I. To show the effect of concentration on absorption, data from three subjects in Table I are plotted in relation to geometric mean concentrations in Figs. 3–5. Examples of the fit of proximal and distal data to Lineweaver-Burk plots (reciprocal of rate vs. reciprocal of concentration) are shown in Figs. 6 and 7. Kinetic data are summarized in Table II.
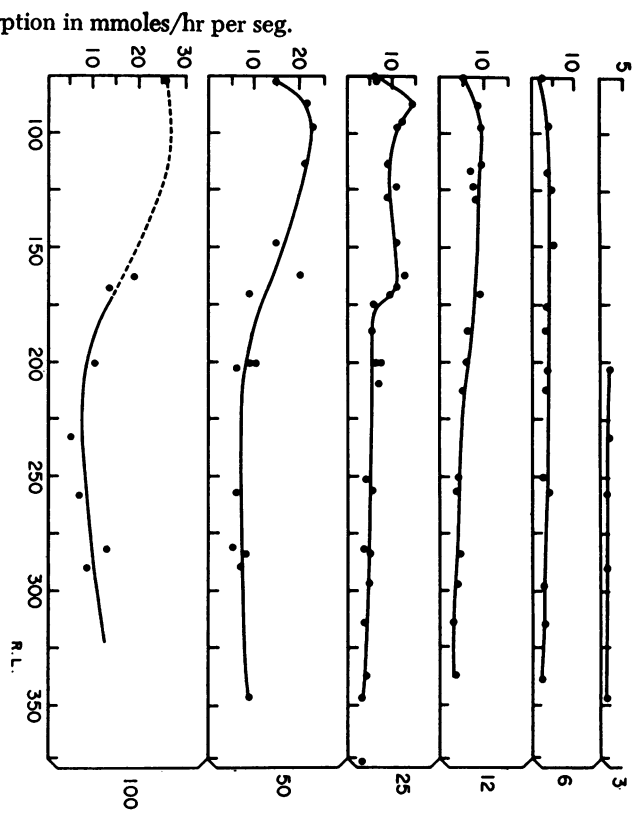
## DISCUSSION

In normal subjects, methionine absorption proceeded at a higher rate in the proximal than in the distal small intestine (Fig. 1), especially at higher methionine concentrations. In contrast, patients with sprue had a zone of low absorption proximally (Fig. 2), where mucosal damage was presumably greatest, and a zone of higher absorption distally. The absorption profile of the small intestine showed individual variations in both normal subjects and patients with sprue. The zone of transition from high proximal absorption rates

was nearer the nares in B. J. and M. R. than in R. L. H. E. showed only a minimal gradient, with very distal transition. In all subjects, the absorption rate in relation to intraluminal concentration showed rate-limiting kinetics in the proximal and distal small intestine (Table I, Figs. 3–5). The $K_m$ and $V_{max}$ data (Table II, Figs. 6 and 7) showed marked differences between the proximal and distal small intestine. In normal subjects, the proximal $K_m$ values ranged from 12 to 28 and the distal from 2.0 to 5.7, depending on concentration range used for calculation. In accord with the high proximal and low distal methionine absorption rates, the proximal $V_{max}$ (16–33 mmoles/hr per segment) was much higher than the distal (5–6 mmoles/hr per segment).
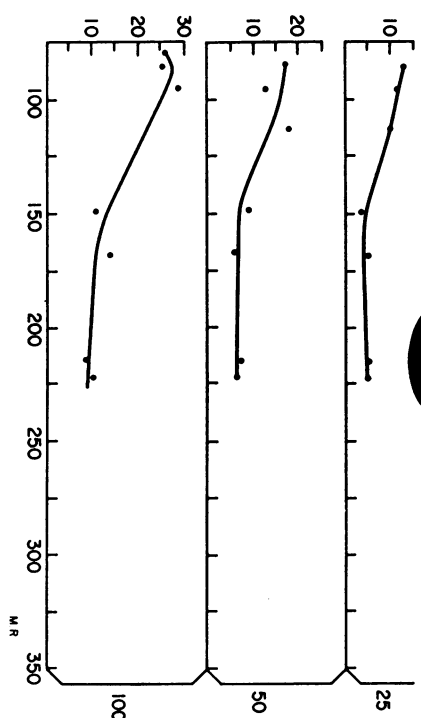
In obtaining $K_m$ and $V_{max}$, it is essential that absorption rates be measured at concentrations that define the rate curve, i.e., both below and above $K_m$. The number of higher concentrations from the flat portion of the curve that are used must be limited, otherwise bias is introduced.
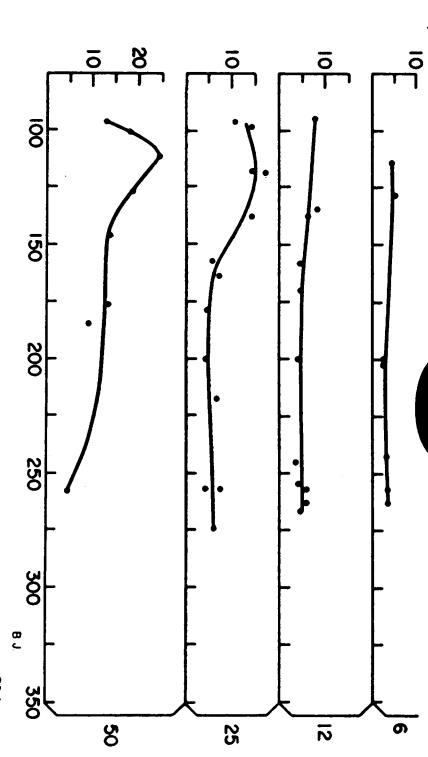
Absorption in mmoles/hr per seg.
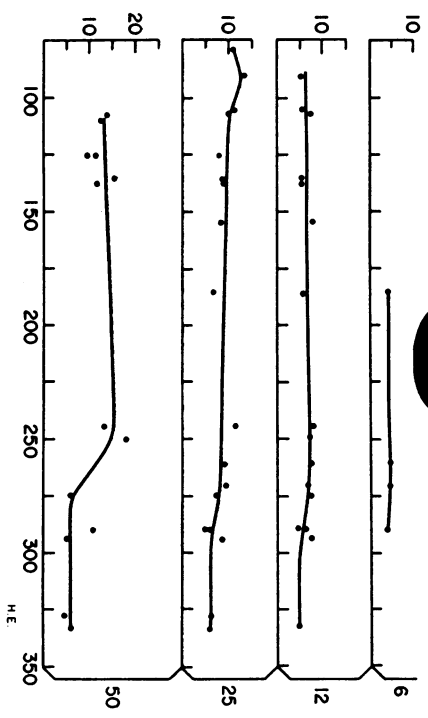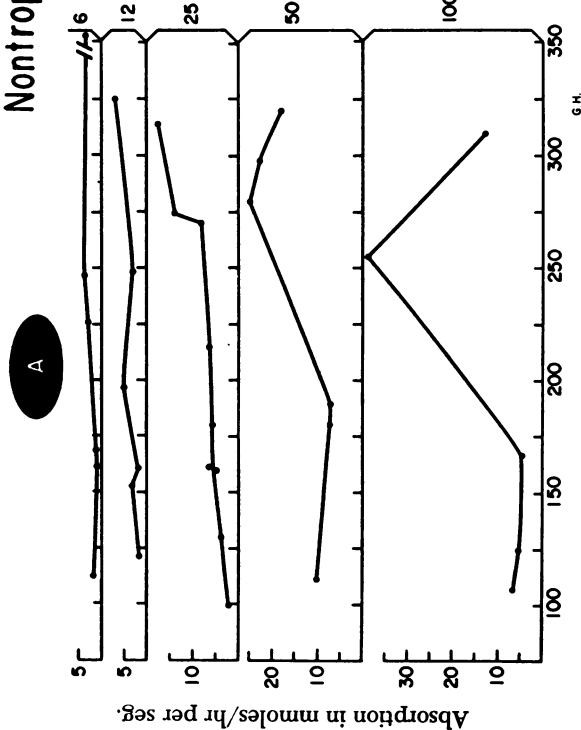
Normal Subjects

Distance from Nares, cm

Initial Concentration, mmoles/liter
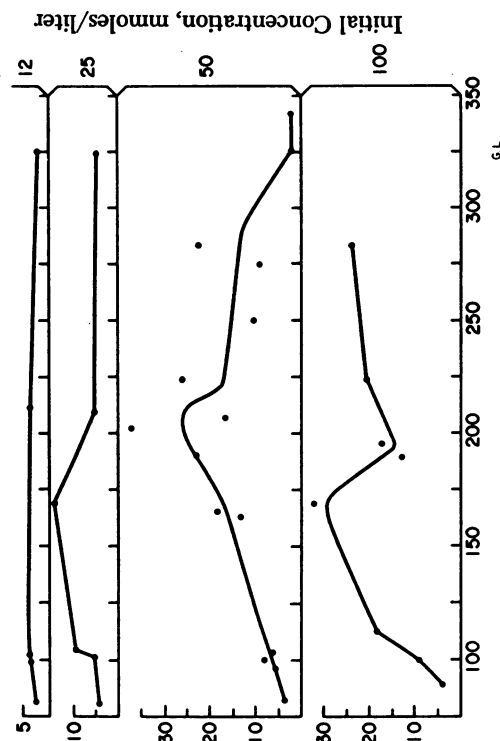
FIGURE 1  Methionine absorption in relation to small intestinal site. Absorption rate (mmoles/hr per segment) on the left vertical axis is plotted at the varying methionine concentrations (3–100 mmoles/liter) on the right vertical axis. Small intestinal perfusion site in distance from the nares in centimeters is on the horizontal axis. Each point is the mean of three to four samples. (A) Data are derived from a total of 258 samples. At low concentration (3 and 6 mmoles/liter) absorption was independent of gut site. As concentration was raised from 12 to 100 mmoles/liter, proximal absorption became progressively greater than distal absorption. (B) Data are derived from 71 samples and show the proximal-distal gradient. (C) Data are derived from 138 samples. The proximal-distal gradient was lacking with the 6 and 12 mmoles/liter of methionine but was present with the 25 and 50. (D) Data are derived from 266 samples.

Nontropical Sprue



FIGURE 2  Methionine absorption in relation to concentration and gut site in nontropical sprue. (A) In contrast to the pattern in normal controls (Fig. 1), proximal rates were lower than distal rates. Data are derived from 107 samples. (B) The proximal rates are low and the highest rates are in the mid–small intestine. Data are derived from 106 samples.

## TABLE II

*Kinetic Constants for L-Methionine Absorption*

| Subjects | $K_m$ | $V_{max}$ | Concn range* |
|---|---|---|---|
| | *mmoles/ liter* | *mmoles/hr per segment* | *mmoles/ liter* |
| **Proximal** | | | |
| **Normal** | | | |
| R. L. | 27.6 | 32.8 | 6–100 |
| M. R. | 24.5 | 30.1 | 25–100 |
| B. J. | 13.7 | 24.5 | 6– 50 |
| H. E. | 11.6 | 15.8 | 12– 50 |
| **Sprue** | | | |
| G. H. | 6.1 | 5.6 | 3–100 |
| G. L. | 7.0 | 7.2 | 6–100 |
| **Distal** | | | |
| **Normal** | | | |
| R. L. | 5.7 | 6.3 | 3– 50 |
| | 4.4 | 5.4 | 3– 25 |
| | 2.8 | 4.7 | 6– 25 |
| B. J. | 2.9 | 6.4 | 3– 50 |
| | 2.6 | 5.9 | 3– 25 |
| H. E. | 2.0 | 6.4 | 6– 50 |
| **Sprue** | | | |
| G. H. | 15.4 | 24.7 | 6–100 |
| G. L. | 48.3 | 34.9 | 12–100 |

\* Absorption rates over this initial concentration range were used for calculating $K_m$ and $V_{max}$ because they appeared to give the best estimate. Inclusion of a wider concentration range did not alter the relative values greatly and appeared to introduce artifact.

Under steady-state conditions, absorption at high concentrations may not be great enough to give reliable rates, because the difference between initial and final concentrations is too small. The converse problem arises at low concentrations when absorption rates are too high. It is difficult to relate rate to a specific concentration, because concentration changes so greatly. For this reason, $K_m$ and $V_{max}$ values at several concentration ranges are given for R. L. and B. J. (Table II, Distal).

The contrasting $K_m$ values between the proximal and distal small intestine in normal subjects suggest differences in transport behavior of methionine consistent with differences in carrier function. The transport of methionine (and of neutral amino acids generally) is heterogeneous in the Ehrlich ascites tumor cell (2). The interactions among neutral amino acids for uptake by the cells showed that affinities clustered into two
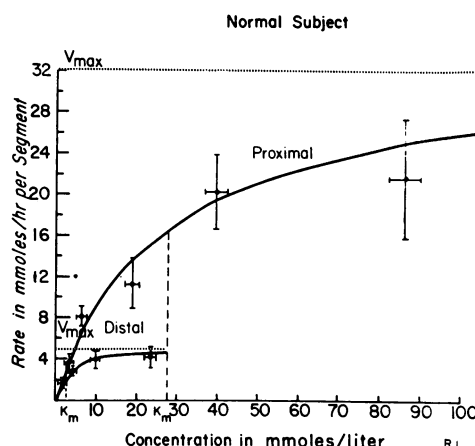


FIGURE 3 Methionine absorption in relation to concentration in proximal and distal small intestine. Absorption rate (mmoles/hr per segment) on the vertical axis is plotted at the geometric mean of initial and final concentration on the horizontal axis. Means and standard deviations of rates and geometric mean concentrations are indicated. $K_m$ (mmoles/liter) is 28 in the proximal and 2.5 in the distal segment, and the respective $V_{max}$ (mmoles/hr per segment) values are 33 and 5. Curves are fitted from $K_m$ and $V_{max}$.

groups. Methionine had a particularly strong dual affinity and appeared in both the lipophilic and hydrophilic groups, although there was some overlap between all members of both groups. More recently, methionine uptake by the Ehrlich cell has been divided into sodium-sensitive and sodium-insensitive components (3). The rat small intestine behaves as if there were a carrier system
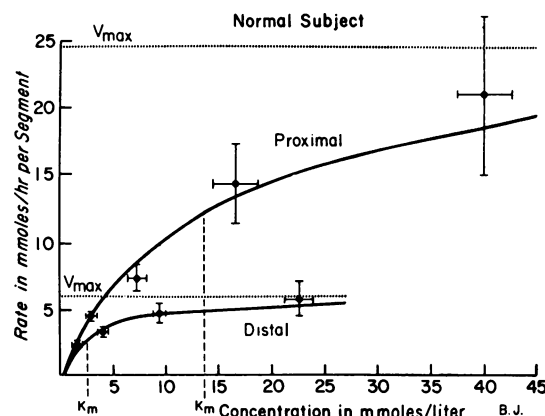


FIGURE 4 Methionine absorption in relation to geometric mean concentration in proximal and distal small intestine. $K_m$ and $V_{max}$ are, respectively, 14 and 25 in the proximal and 2.6 and 6 in the distal segments.
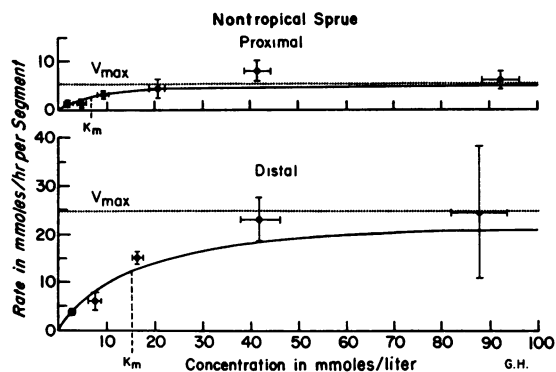
FIGURE 5 Methionine absorption in relation to geometric mean concentration in proximal and distal small intestine in a patient with nontropical sprue. In contrast to normal controls (Figs. 3 and 4), absorption rate, $K_m$, and $V_{max}$ of the distal segments are greater than those of the proximal segments.
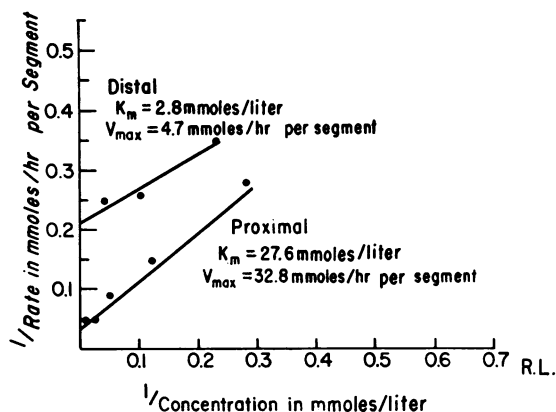


FIGURE 6 Lineweaver-Burk plot of proximal and distal methionine absorption in a normal subject. The reciprocal of the mean rate is plotted on the vertical axis against the reciprocal of the geometric mean concentration on the horizontal axis. Lines are fitted by the method of least squares.

common to methionine, glycine, and proline, but with an additional carrier for glycine and proline (5). However, in the rat, either the middle-fifth or the entire small intestine was used, and different segments were not compared. Thus, our finding of an apparent spatial separation with differences of transport at different loci has not previously been demonstrated.

Although in our studies the functional behavior of the human small intestine is consistent with the operation of two different carriers, we have obtained no information on homogeneity of transport systems at either site. Thus, both hypothetical carriers could be present in different proportions in the two sites, or there could be multiple carriers at each site. It is also possible that the basic amino

acid transport system(s) may be involved in methionine transport. In addition, in our studies, as in all others on methionine transport (4, 6–8), the intraluminal concentrations of methionine were well above those in blood. Therefore, differences in diffusion permeability between the two segments might explain the differences in transport. The high proximal and low distal $V_{max}$ values are consistent with the transport rates actually observed at the two sites. Because of the probability that multiple transport processes are involved, the usual relationship of proportionality between $V_{max}$ and number of carrier sites seen with only one carrier does not apply here.
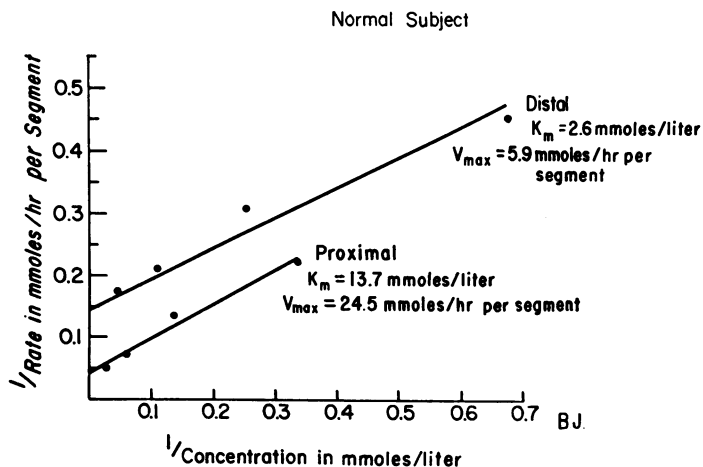


FIGURE 7 Lineweaver-Burk plot of proximal and distal methionine absorption in a normal subject.

Functionally, the contrasting transport characteristics defined by $K_m$ and $V_{max}$ can be described as follows. Proximal transport, as shown by the high $K_m$, was difficult to saturate and required high concentrations of substrate. It promoted rapid absorption of large amounts of methionine (high $V_{max}$) when supplied at high intraluminal concentrations (high $K_m$). Distal transport was saturated at much lower concentrations (low $K_m$) and had a limited capacity (low $V_{max}$). At low concentrations, it would move more methionine per unit time than would the proximal, when both were operating at the same substrate concentration. This proximal-distal kinetic pattern is consistent with optimal small intestinal function. Thus, during feeding, when both exogenous and endogenous (19) amino acids entered the proximal gut at high concentrations, they would be rapidly transported out of the lumen by a downhill process. The absorptive mechanism in the distal gut would remove the low concentrations of amino acid left by the action of the proximal system. Because of the probable reversibility of the transport system for neutral amino acids (20), it is conceivable that the distal mechanism may reabsorb blood amino acids that have leaked into the gut lumen, even though blood amino acid levels are normally quite low.

Determinations of amino acid $K_m$ values in the human small intestine published since these studies were completed have been confined to the jejunum. Alanine has a longer lipophilic side chain and had a lower $K_m$ (34 and 38 mmoles/liter) than glycine (75 and 77 mmoles/liter) (9). Methionine, with an even longer lipophilic side chain, would be expected to show a still lower $K_m$. We found the mean $K_m$ for methionine in the proximal small intestine to be about 19 mmoles/liter (Table II). The $K_m$ for L-methionine in the entire rat small intestine in vivo was 9.6 mmoles/liter (calculation based on initial concentration) (21). Thus, our distal $K_m$ values are lower than reported observations, but this segment was not specifically investigated in the other studies.

The low proximal absorption rates in sprue could be explained by mucosal damage with loss of villi. The decrease in surface area of the perfused segment would be accompanied by a decrease in methionine carrier. The causes for increased distal absorption in sprue are unknown. The surface area of the perfused segment is a primary determinant of absorption. If the effective absorbing surface of distal segments in patients with sprue were greater than in normal subjects, other factors being equal, absorption rate would be greater. Motility is decreased in sprue (22). If this decreased motility caused pooling of perfusate and retrograde flow, the perfused segment would be longer than in normal subjects. Thus, in the approximate steady state in sprue, additional surface area would be recruited by retrograde flow, and contact time would be longer than in normal subjects. Since the distal mucosa is nearly normal in sprue (23), total methionine carriers in the longer perfused segment of such patients might be greater than in the normal subject and could account for the increased distal absorpton. Cortisol absorption in the distal small gut in sprue may also be greater than normal (12). Decreased motility and retrograde flow would also explain increased cortisol absorption. For cortisol, the increase in segment length would provide increased surface area for diffusion.

Absorption rates of the most proximal segments (75–80 cm) were frequently lower than slightly more distal studies (Fig. 1: 6, 12, 25, 50 mmoles/liter in R. L.; 25, 50 mmoles/liter in B. J.). Although we have noted this same phenomenon in cortisol and glucose absorption studies (12), we are not certain of its significance. A definite depression of absorption was seen in this zone when there was entry of gastric contents, as was detected by the fall in sample pH to below 6.0. Such samples were excluded from this investigation, but the absorption-decreasing effect may have been present without a pH change.

## ACKNOWLEDGMENTS

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