# Comparative Nutritional Value for Amino Acids, Oligopeptides and Soybean Protein

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**In recent years our view on the absorption of protein digestion products has undergone a radical change. We now believe that intralumen hydrolysis of intake proteins to free amino acids is only partial. It has thus been hypothesized that partial protein hydrolysates or oligopeptide mixtures are nutritionally superior to corresponding amino acid mixtures. With this consideration for a background, we developed a sophisticated technique of protease-catalyzed modification of soy protein to produce enzymatically modified proteins (EMP) having different levels of covalently attached methionine. We also carried out feeding tests with protein-malnourished rats, with the result that for their recovery from a state of malnutrition, an EMP containing peptide-bound methionine at 3% was significantly more effective than the corresponding amino acid mixture and even the soy protein itself. Discussions stress the importance of applying such a technique to production of an oligopeptide rather than a free amino acid nitrogen source as a foodstuff for therapeutic use.**

Recent research on protein nutrition has elucidated the digestion and absorption mechanism involving peptides. It is now believed that dipeptides and tripeptides formed by intralumen hydrolysis of intake proteins are mostly absorbed prior to further hydrolysis to free amino acids (1-5). Presumably, therefore, supplementation of low-quality proteins is more effective when essential amino acids are used as peptides rather than as free amino acids (6,7). To test this hypothesis, we must examine differences in digestion and absorption features, protein efficiency ratio (PER), and many other nutritional indices between an oligopeptide mixture (OPM) and the corresponding free amino acid mixture (AAM).

In this study we undertook experiments to examine nutritional effects of supplemental peptide-bound methionine with a soy protein isolate (SPI) and those of OPM products that may be usable as alimentary diets for recovery from a state of protein malnutrition.

## EXPERIMENTAL

*Preparation and characterization of enzymatically modified proteins.* To prepare the peptide-bound methionine we utilized a papain-catalyzed reverse reaction which could proceed under an unusual condition (8-16), and then we compared the resulting enzymatically modified protein (EMP) with the AAM of the same amino acid composition. A recrystallized preparation of papain (EC 3.4.22.2) was used to modify SPI with covalent incorporation of L-methionine. In this case, methionine was used in ethyl ester form (8). The conditions for the modification were set according to the previous paper (10). These were as follows: medium, 1 M carbo-

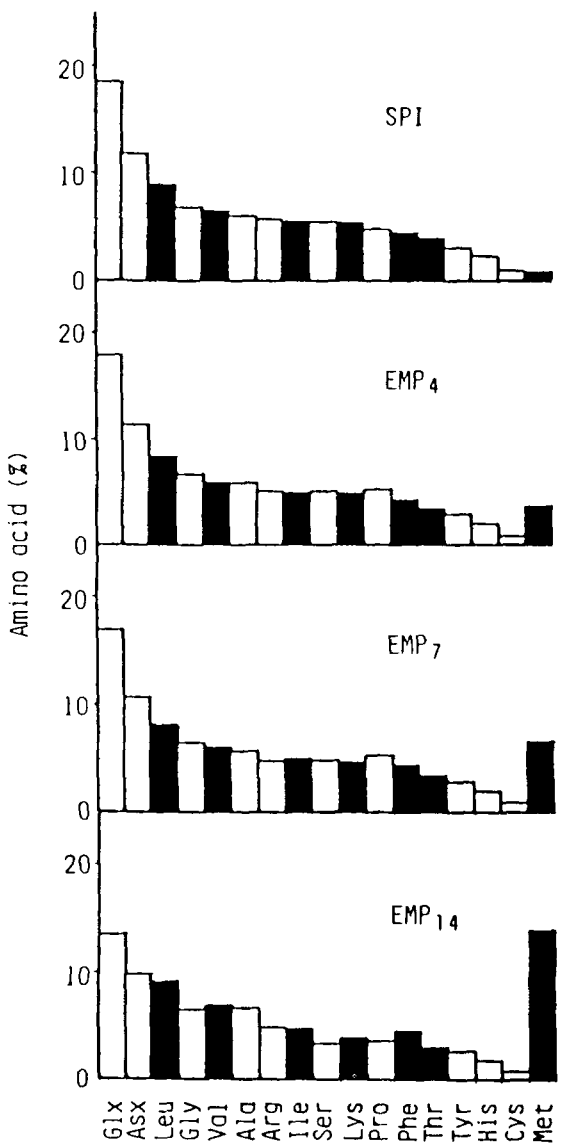


FIG. 1. Amino acid patterns of SPI and a series of EMP products. Suffixed figures stand for approximate percentage of methionine contents of the EMP products. Filled columns indicate essential amino acids.

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nate (pH 9) containing 10 mm L-cysteine; concentration of SPI in the medium, 25%; concentration of papain in the medium, 0.25%; incubation temperature, 37 C, and incubation time, 2 hr. After the incubation the mixture was dialyzed against water and an EMP product was obtained by lyophilization of the non-diffusible fraction. The methionine content of the EMP was adjusted by controlling the amount of methionine ethyl ester added to the reaction medium. Thus, we obtained a series of EMP products whose methionine levels were altered progressively (Fig. 1). Chemical analyses showed that every EMP product was a peptide mixture having a molecular weight distribution in the range of 2,000–12,000 daltons with an average at approximately 4,000 daltons. The positions of the methionine molecules incorporated were estimated by treatment of $EMP_7$ (Fig. 1) with exopeptidases. The treatment with leucine aminopeptidase indicated that no significant amount of methionine residues existed at or near the N-terminal position. On the other hand, by treating this protein with carboxypeptidase A, we found that the methionine residues were mostly located at or near its C-terminal position (17). From these results and other chemical analyses it was estimated that these methionine molecules existed in the form of an oligomer at the C-terminal position.

*Digestion and absorption studies with EMP.* Feeding tests were carried out with seven-week-old male rats (Wistar strain) weighing approximately 200 g each which had been imprinted for meal feeding. Two kinds of diets were prepared in accordance with Harper's composition (18); one was based on an EMP containing methionine at a 3% level ($EMP_3$) and the other on the simulating AAM formulated similarly. Each of the diets was also set at a nitrogen level of 1.5% (Table 1). The diets were administered to rats by meal feeding per os, and portal blood samples were taken every hour thereafter. At the same time, the contents of their small intestinal tracts were washed out with water and, immediately, treated with 10% trichloroacetic acid. Subsequently, the resulting supernatant was taken and fractionated on the basis of molecular weight by membrane filtration to obtain fractions of lower than 500 daltons, 500–1,000 daltons and 1,000–2,000 daltons. We also carried out another experiment to enhance the nutritional value of SPI by adding $EMP_{10}$, which stands for an EMP containing methionine at a 10% level. Diets were prepared according to Harper's composition (18) with seven different methionine levels varying in the range of 0.1–0.4% (Fig. 2), where nitrogen levels were all adjusted at 1.5%. Each of the diets was fed ad libitum to four-week-old male rats (Wistar strain) weighing approximately 80 g each. After the feeding of the rats was conducted for four weeks, their food intakes and body weight gains were measured to obtain PER values.

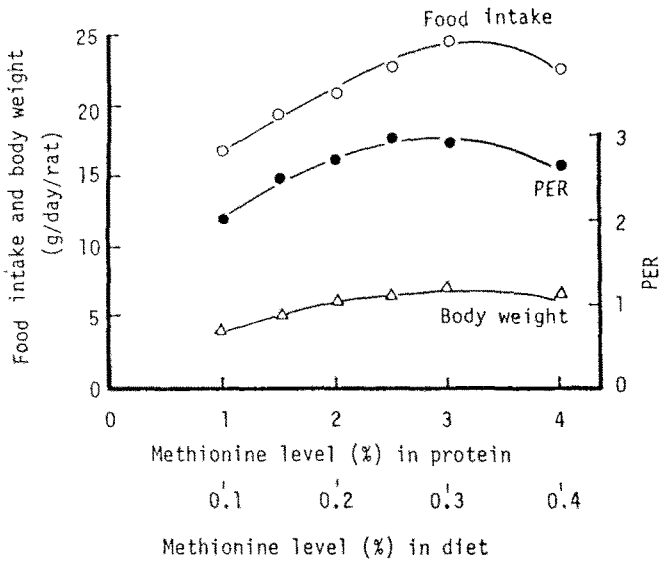


**FIG. 2. Food intakes, weight gains and PER values observed for rats fed the SPI-$EMP_{10}$ formulas having different methionine levels.**

TABLE 1

**Diet Composition**

| Ingredient | Composition (weight percent) | |
|---|---|---|
| | $EMP_3$ diet | AAM diet |
| $EMP_3$ | 10.5[a] | – |
| AAM | – | 9.7[a] |
| Corn Starch | 69.15 | 69.95 |
| Sucrose | 10.0 | 10.0 |
| Soybean oil | 5.0 | 5.0 |
| Mineral mixture[b] | 4.0 | 4.0 |
| Cellulose powder | 1.0 | 1.0 |
| Choline chloride | 0.15 | 0.15 |
| Vitamin mixture[c] | 0.20 | 0.20 |

[a]Equivalent to 1.5% nitrogen.

[b]Amounts of salts in 100 g of the mineral mixture: $CaCO_3$, 29.29 g; $CaHPO_4 \cdot 2H_2O$, 430 mg; $KH_2PO_4$, 34.31 g; NaCl, 25.06 g; $MgSO_4 \cdot 7H_2O$, 9.98 g; $Fe(C_6H_5O_7) \cdot 6H_2O$, 623 mg; $CuSO_4$, 156 mg; $MnSO_4 \cdot H_2O$, 121 mg; $ZnCl_2$, 20 mg; KI, 0.5 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 2.5 mg; and $Na_2SeO_3 \cdot 5H_2O$, 1.5 mg.

[c]Amounts or units of vitamins in 100 g of the vitamin mixture: thiamine hydrochloride, 100 mg; riboflavin, 100 mg; niacinamide, 500 mg; calcium pantothenate, 400 mg; pyridoxine hydrochloride, 100 mg; folic acid, 10 mg; menadione, 10 mg; d-biotin, 4 mg; vitamin $B_{12}$, 0.6 mg; ascorbic acid, 1 g; vitamin E acetate, 8 g; vitamin A acetate, 80,000 units; and vitamin $D_2$, 8,000 units.

*Preparation and characterization of oligopeptide mixture (OPM).* We prepared an OPM product by hydrolyzing $EMP_3$ with trypsin. $EMP_3$ (100 g) was dissolved in water (5 l) and adjusted to pH 7.8 with NaOH. The solution was incubated with a recrystallized preparation of trypsin (100 mg) at 37 C for 15 min. Absolute ethanol (10 l) was added to stop the enzymatic reaction and the resulting 66.6% ethanol solution allowed to stand for 6 hr at room temperature. A precipitate formed and was removed by centrifugation at 4,000 rpm for 10 min; the supernatant was saved. The precipitate (42 g on a dry-matter basis) was submitted to a repetition of

TABLE 2

**Amino Acid Patterns of SPI and $OPM_3$**

| Amino acid | SPI (g amino acid/16 g nitrogen) | $OPM_3$ (g amino acid/16 g nitrogen) |
|---|---|---|
| Asx | 11.13 | 12.12 |
| Thr | 3.65 | 3.69 |
| Ser | 4.81 | 4.91 |
| Glx | 20.42 | 21.44 |
| Pro | 5.66 | 2.43 |
| Gly | 3.97 | 4.45 |
| Ala | 4.33 | 4.62 |
| Cys | 1.04 | 1.10 |
| Val | 5.04 | 5.20 |
| Met | 1.07 | 3.11 |
| Ile | 4.78 | 5.52 |
| Leu | 7.05 | 7.59 |
| Tyr | 3.79 | 3.57 |
| Phe | 5.25 | 5.92 |
| Trp | 0.98 | 0.99 |
| Lys | 5.72 | 5.42 |
| His | 2.65 | 2.02 |
| Arg | 7.22 | 5.52 |

the tryptic hydrolysis followed by the ethanol treatment to obtain a second supernatant. The first and the second supernatants were pooled prior to evaporation of the ethanol in vacuo. Lyophilization of the residue gave an OPM product in a yield of 87 g. Amino acid analysis and gel chromatography demonstrated that the OPM product had an amino acid composition shown in Table 2 and an average molecular weight of ca. 900 daltons as shown in Figure 3; no appreciable amounts of free amino acids were present. According to the methionine content (ca. 3%) of this product, it was encoded as $OPM_3$.

*Feeding tests with normal and malnourished rats.* By simulating the amino acid composition of $OPM_3$ (Table 2) we prepared a free amino acid mixture which is encoded as $AAM_3$. On the other hand, $SPI_3$ was prepared by mixing free methionine with SPI so as to reach a level of 3% with respect to total methionine content. With these comparable nitrogen sources ($SPI_3$, $OPM_3$ and $AAM_3$) at a nitrogen level of 1.5% each, we formulated three different diets of Harper's composition (18). In a feeding test with normal subjects, male rats (Wister strain) aged 4 weeks, weighing about 80 g each, were fed these experimental diets for 3 weeks. For preparation of protein-malnourished rats as abnormal male rats aged 6 weeks, weighing about 170 g each, were depleted by feeding with a protein-free diet for 2 weeks until their average body weight decreased to approximately 140 g and symptoms of anemia appeared. The malnourished rats were then fed the experimental diets for the following 3 weeks.

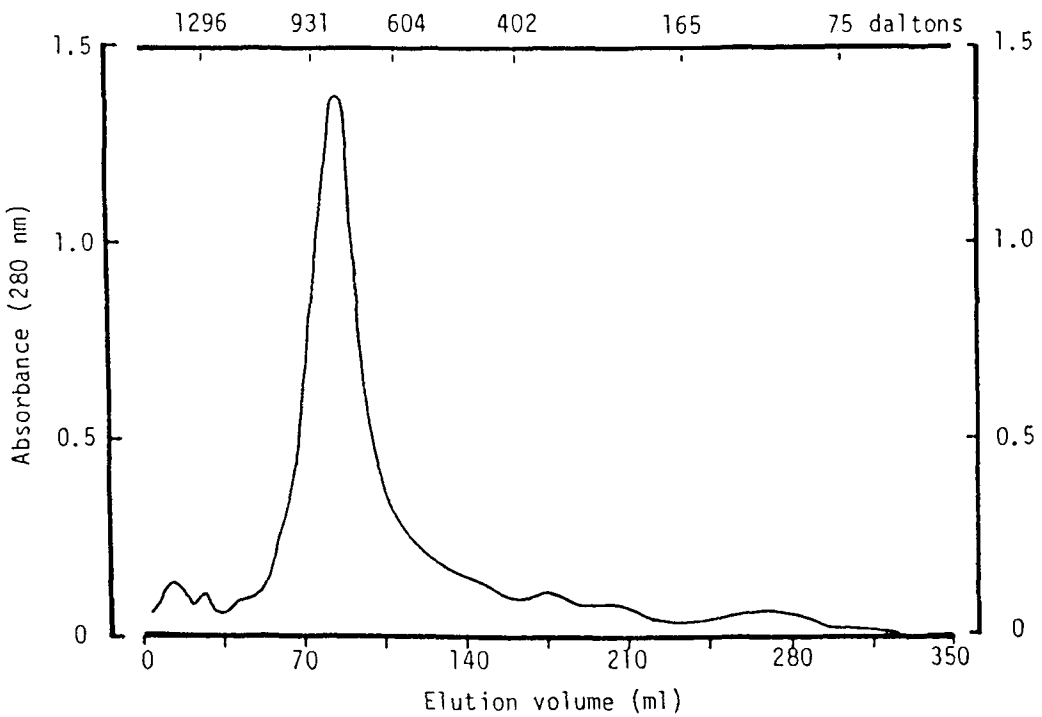


**FIG. 3. Chromatography of $OPM_3$ on Bio-Gel P-2 (2 × 100 cm). The elution was made with 0.2 M phosphate, pH 8, at room temperature. The following markers with known molecular weights (MW) were used: human angiotensin I (MW 1296), human angiotensin III (MW 931), antipain (MW 604), triglutamic acid (MW 402), L-phenylalanine (MW 165), and glycine (MW 75).**

## RESULTS AND DISCUSSION

*Digestion and absorption of $EMP_3$.* It was observed that free amino acids in the portal vein of rats fed the $EMP_3$ diet reached a maximum at 2 hr after feeding and decreased gradually thereafter. The concentration of free methionine in the portal vein also showed a similar time-course profile. Portal amino acid concentrations of rats fed the simulated AAM diet were almost similar to those observed with the rats of the $EMP_3$ diet group. These results indicate that the covalently attached methionine occurring in $EMP_3$ is well bioavailable. However, there was a difference between the EMP diet group and the AAM diet group in terms of the amount of small intestinal nitrogen content. For the $EMP_3$ diet group we observed a bell-shaped profile showing that the small intestinal nitrogen content reached a maximum at 2 hr after feeding. The amount of total methionine, i.e., free methionine plus peptide-bound methionine, showed a similar time-course profile. However, free methionine was at a very low level over the observation period, representing only 15% of total methionine even at 2 hr after feeding. In particular, the free methionine amount observed for the AAM diet group was six times as high as that observed for the $EMP_3$ diet group at 1 hr after feeding, whereas in the portal vein this relationship was reversed. Fractionation experiments with the small intestinal content of $EMP_3$ diet group at 2 hr after feeding showed that it was comprised of molecular weight fractions of higher than 2,000 daltons (18.3%), 1,000-2,000 daltons (21.5%), 500-1,000 daltons (21.1%) and lower than 500 daltons (26.3%). It was also found that a major part of the methionine occurred in the fraction of lower than 500 daltons. Probably, therefore, the methionine existed mostly in the form of oligopeptides.

*Supplementation of soy protein with $EMP_{10}$.* The feeding test with rats to examine a supplementary effect of $EMP_{10}$ gave the following result. The maximum PER value was observed when rats were fed the diet containing the peptide-bound methionine at 0.25-0.30% (Fig. 2). In considering that, in general, the PER value of SPI is maximized when rats are fed SPI whose methionine level is enhanced up to 3-4% by addition of free methionine (20), the result that the methionine level of 2.5-3.0% maximized the PER of SPI (Fig. 2) may suggest a superior nutritional effect of the peptide-bound methionine occurring in $EMP_{10}$. Though the most recently published information (21) suggests that soy protein itself needs no supplementary methionine, it may still be useful to apply our method for supplementation to other low-quality proteins for human consumption. It may also be beneficial that $EMP_{10}$ as well as other EMP products is low in flavor despite its high methionine content.

*OPM as a foodstuff for therapeutic use.* It is likely that OPM, when administered per os, behaves differently from corresponding AAM in some respects in absorptive and postabsorptive stages (6-7, 22-24), but little is known of any potential difference between them in terms of overall nutritional parameters including body weight gain and PER. For clear detection of such a difference between the nutritional effect of OPM and that of AAM, it may sometimes be preferable to use abnormal animals, e.g., malnourished animals, as subjects for investigation. To obtain information about this point, we carried out a feeding test with normal and protein-malnourished rats where they were fed the $OPM_3$ diet as a test diet and the $SPI_3$ and $AAM_3$ diets as controls. Rats of the different diet groups accepted their diets well. During the feeding period (3 weeks) we measured food intakes and body weight gains to obtain PER values and dietary and fecal nitrogen amounts to obtain digestibilities. We also examined the rats for their blood chemistry involving glutamine-oxoacid transaminase (GOT) and glutamine-pyruvate aminotransferase (GPT) as physiological parameters (25). The results are summarized in Table 3. Both for normal and for malnourished rats, $SPI_3$ was significantly less digestible than $OPM_3$. However, both normal and malnourished rats utilized $SPI_3$ and $OPM_3$ with similar efficiency. In the case of malnourished rats, the PER value observed for $OPM_3$ was significantly higher than that observed for $AAM_3$. It was also likely from the data on GPT that $OPM_3$ surpassed $AAM_3$ in blood physiology. These results suggest that $OPM_3$ could be utilized similarly to or more efficiently than SPI from which it was derived and also that $OPM_3$ is generally superior to $AAM_3$. It is thus expected that $OPM_3$ would be used as an oligopeptide rather than a free amino acid nitrogen source for faster recovery of unhealthy subjects under therapeutic care.

TABLE 3

**Performance of a Test with Normal and Protein-Malnourished Rats Fed $SPI_3$-, $OPM_3$- and $AAM_3$-Diets for Three Weeks**

| State of rats | Number of rats | Nitrogen source | Digestibility (%) | Body weight gain (g/day) | PER | GOT (Karmen units) | GPT (Karmen units) |
|---|---|---|---|---|---|---|---|
| Normal | 5 | $SPI_3$ | 87.0 } a | 5.84 } a | 2.96 | 66 | 8.3 |
| Normal | 5 | $OPM_3$ | 92.3 } a | 6.24 } a | 3.12 } a | 55 | 11.0 |
| Normal | 5 | $AAM_3$ | 92.0 | 5.81 } a | 2.72 | 69 | 14.0 |
| Malnourished | 5 | $SPI_3$ | 87.0 } a | 8.10 } a | 3.88 | 68 | 12.0 |
| Malnourished | 5 | $OPM_3$ | 91.1 } a | 8.67 } a | 3.97 } a | 55 | 11.0 |
| Malnourished | 5 | $AAM_3$ | 92.6 | 7.82 } a | 3.49 } a | 65 | 13.5 } a |

[a]Students' t-test. $P < 0.05$.

## REFERENCES

1. Adibi, S.A., *J. Clin. Invest. 50*:2266 (1971).
2. Adibi, S.A., and D.W. Marcer, *Ibid. 52*:1586 (1973).
3. Matthews, D.M., and S.A. Adibi, *Gastroenterol. 71*:151 (1976).
4. Matthews, D.M., *Ibid. 73*:1267 (1977).
5. Matthews, D.M., in *Peptide Transport and Hydrolysis* (Ciba Foundation Symposium 50), Elsevier Excepta Medica, North-Holland, 1977, p. 5.
6. Arai, S., *Jap. J. Food Nutr. 31*:247 (1978).
7. Hara, H., R. Funabiki, M. Iwata and K. Yamazaki, *J. Nutr. 114*:1122 (1984).
8. Fujimaki, M., S. Arai and M. Yamashita, in *Food Proteins: Improvement through Chemical and Enzymatic Modification*, edited by R.E. Feeney and J.R. Whitaker, American Chemical Society, Washington, D.C., 1977, p. 156.
9. Yamashita, M., S. Arai, Y. Imaizumi, Y. Amano and M. Fujimaki, *J. Agric. Food Chem. 27*:52 (1979).
10. Yamashita, M., S. Arai, Y. Amano and M. Fujimaki, *Agric. Biol. Chem. 43*:1065 (1979).
11. Arai, S., M. Yamashita and M. Fujimaki, *Ibid. 43*:1069 (1979).
12. Arai, S., M. Watanabe, *Ibid. 44*:1979 (1980).
13. Watanabe, M., H. Toyokawa, A. Shimada and S. Arai, *J. Food Sci. 46*:1467 (1981).
14. Watanabe, M., A. Shimada, E. Yazawa, T. Kato and S. Arai, *Ibid. 46*:1738 (1981).
15. Toiguchi, S., S. Maeda, M. Watanabe and S. Arai, *Agric. Biol. Chem. 46*:1587 (1982).
16. Watanabe, M., and S. Arai, in *Modification of Proteins: Food, Nutritional and Pharmacological Aspects*, edited by R.E. Feeney and J.R. Whitaker, American Chemical Society, Washington, D.C., 1982, p. 199.
17. Aso, H., H. Kimura, M. Watanabe and S. Arai, *Agric. Biol. Chem. 49*:1649 (1985).
18. Harper, A.E., *J. Nutr. 68*:405 (1959).
19. Arai, S., H. Aso and H. Kimura, *Agric. Biol. Chem. 47*:2115 (1983).
20. Inoue, G., K. Kishi and I. Yagi, *J. Nutr. Sci. Soy Protein 1*:6 (1980).
21. Report of a Joint FAO/WHO/UNU Expert Consultation: *Energy and Protein Requirements* (Technical Report Series 724), World Health Organization, Geneva, 1985, p. 64.
22. Silk, D.B.A., T.C. Marrs, J.M. Addison, D. Burston, M.L. Clark and D.M. Matthews, *Clin. Sci. Mol. Med. 45*:715 (1973).
23. Imodi, A.R., and R.P. Stradley, *J. Nutr. 104*:793 (1974).
24. Silk, D.B.A., P.D. Fairclough, M.L. Clark, J.E. Hegarty, T.C. Marrs, J.M. Addison, D. Burston, K.M. Clegg and D.M. Matthews, *J. Parenteral & Enteral Nutr. 4*:548 (1980).
25. Karmen, A., *J. Clin. Invest. 34*:131 (1955).

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