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Utilization of alanyl-L-glutamine and glycyl-L-glutamine during long-term parenteral nutrition in the growing rat

M. Neuhäuser-Berthold and **H. Eufinger**

Fachbereich Haushalt und Ernährung, Fachhochschule Fulda, Federal Republic of Germany

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Summary. Utilization of intravenously administered alanyl-L-glutamine and glycyl-L-glutamine as a source of glutamine was tested in growing rats receiving total parenteral nutrition for 15 days. In experiment A the two peptides were compared to each other, in experiment B alanyl-L-glutamine was compared to an equimolar mixture of free alanine and glutamine, and in experiment C glycyl-L-glutamine was tested against an equimolar mixture of free glycine and glutamine.

There was no difference of statistical significance in weight gain or nitrogen balance between the respective groups participating in the 3 experiments. Plasma levels of free glutamine were similar during infusion with alanyl-L-glutamine or glycyl-L-glutamine. The daily urinary excretion rate recorded for alanyl-L-glutamine was 3.7% and for glycyl-L-glutamine 4.3% of the infused amount. The results show that both peptides are utilized for protein synthesis and growth to approximately the same as the corresponding free amino acids.

Keywords: Amino acids – Alanyl-L-glutamine – Glycyl-L-glutamine – Parenteral nutrition

Introduction

During starvation, metabolic stress, and catabolism amino acids are shuttled from muscle to splanchnic tissues, glutamine (Gln) being one of the most important in point of quantity. Consequently, a considerable decrease of intracellular Gln concentrations in muscle is observed (Aulick and Wilmore, 1979; Fürst et al., 1979; Askanazi et al., 1980; Muhlbacher et al., 1984) which appears to be dependent on the severity of stress and obviously cannot be influenced by conventional TPN (Roth et al., 1982; Gamrim et al., 1991; Fürst, 1985). Uptake by the visceral tissues exceeds the amounts released by muscle, resulting in a

decline of plasma levels. Due to its instability Gln is not present in standard parenteral solutions. Gln is a major oxidative fuel for the small intestine (Windmueller, 1982). It has been shown that parenteral nutrition results in mucosal atrophy of the small intestine (Johnson et al., 1975) and that preservation of small bowel mucosa can be achieved by using Gln enriched solutions (Jacobs et al., 1988; Hwang et al., 1986). Furthermore, there is some evidence that glutamine may be essential for normal function of lymphocytes, macrophages, and thymocytes which are all important: immunologic cells (Ardawi, 1988; Newsholme et al., 1988; Caldwell, 1989). At present the introduction of stable Gln derivates like acetyl-L-glutamine (Ac-Gln) (Neuhäuser and Bässler, 1986), alanyl-L-glutamine (Ala-Gln) (Fürst et al., 1987), and glycyl-L-glutamine (Gly-Gln) (Adibi, 1987) as a Gln source in amino acid solutions for parenteral nutrition is being discussed. From infusion studies with Ac-Gln in rats and in man it was concluded that Ac-Gln is efficiently utilized (Neuhäuser-Berthold et al., 1988d; Magnusson et al., 1987). Nevertheless, urinary losses of Ac-Gln during infusion were considerable both, in the rat and in man. In this study we investigated the utilization of Ala-Gln and Gly-Gln during long-term parenteral nutrition in the growing rat.

Material and methods

Animals

Young male Sprague-Dawley rats were used in this study. Upon arrival, the animals were housed individually in wire cages in a room with constant temperature and humidity and regular intervals of light and darkness. The rats were then about 36 days of age and weighed approximately 140 g. One day after arrival, the rats were provided with a small harness, to which they were allowed to adapt for six days. The purpose of the harness was to protect the infusion catheter, which was inserted into the jugular vein on the eighth day after arrival.

Experimental design

The rats were allowed a 3 day recovery period after surgery. Until that time, the rats had free access to water and were given a diet low in fiber content (Kontroll-Diät C-1000, Altromin, Lage, FRG). On the 4th day after operation intravenous alimentation was started according to a method described elsewhere (Neuhäuser et al., 1984). The intravenous feeding regimen consisted of a complete solution including carbohydrates, amino acids, fat, electrolytes, vitamins, and trace elements. Only minimum requirements for nitrogen (1.0 g $N \times kg^{-1} \times day^{-1}$) were given so that alterations in the amino acid solution would reflect changes in weight gain and nitrogen balance. In three consecutive experiments the rats were randomly assigned to two groups respectively and transferred to metabolic cages. All animals were given 0.6 g $N \times kg^{-1} \times day^{-1}$ as essential amino acids. In experiment A additional 0.4 g $N \times kg^{-1} \times day^{-1}$ was given as Ala-Gln (solution l) to group I ($n = 6$) and as Gly-Gln (solution 2) to group II ($n = 5$). In experiment B additional 0.4 g $N \times kg^{-1} \times day^{-1}$ was given as Ala-Gln (solution 1) to group III ($n = 5$) and as an equimolar mixture of free alanine (Ala) and Gln (solution 3) to group IV ($n = 4$). In experiment C group V ($n = 6$) received additional 0.4 g $N \times kg^{-1} \times day^{-1}$ as Gly-Gln (solution 2), while group VI ($n = 5$) was given an equimolar amount of free glycine (Gly) and Gln (solution 4). In addition the rats of all groups received per kg body weight and day 2.0 g fat and glucose up to a total of 350 kcal. The nutrient solutions were freshly prepared daily for each animal separately and infused over a period of 22 hours/day. The infusion period lasted 15 days and the first 3 days were allowed for adaptation from oral to intravenous feeding. The model permitted free

movement of the animals throughout the entire study. The investigation was performed with the approval of the local Committee for Care and Use of Laboratory Animals.

Infusion solutions

Carbohydrates were supplied as glucose 40% (Pfrimmer & Co., Erlangen, FRG) and fat was given as Intralipid 20% (Pfrimmer & Co., Erlangen, FRG). Electrolytes, trace elements, and vitamins were added as concentrates to the infusion mixture. A detailed listing of the components is published elsewhere (Neuhäuser et al., 1984). The composition of the essential amino acid solution was based on the nutritional requirements of the laboratory rat. One liter of the 7.5% solution contained 7.8 g L-arginine, 3.8 g L-histidine, 7.3 g L-isoleucine, 9.7 g L-leucine, 7.8 g L-valine, 11.7 g L-lysine, 7.8 g L-methionine, 10.5 g L-phenylalanine, 6.7 g L-threonine, and 2.1 g tryptophan. For the intravenous supply with Ala-Gln or Gly-Gln 10% solutions were used (Pfrimmer & Co., Erlangen, FRG). Equimolar solutions containing 5% of each Ala and Gln or Gly and Gln were prepared daily in sterile water and filtered before use through single use filter units with a pore width of 0.2 μm (Minisart NML, SM 16534, Sartorius, Göttingen, FRG).

Analyses

Weight gain, N-intake, and N-excretion were recorded daily in all animals. Twenty-four hour urine was collected in plastic beakers and concentrated HCl was added for preservation. N-excretion in urine was determined by chemiluminescence (Ward et al., 1980) using an N-analyzer (model 703 B, Antek Instruments, Houston). Urinary losses of Ala-Gln and Gly-Gln and of free amino acids were also determined. After day 15 of the infusion period the rats were anesthesized with ketanest and blood samples were obtained from the vena cava inferior under continuous infusion with the respective nutrient solutions. The blood samples were processed immediately for analysis of amino acids and Ala-Gln and Gly-Gln in plasma. Analysis was performed by ion-exchange chromatography using an automated amino acid analyzer (model LC 5000, Biotronik, Munich, FRG) with ninhydrin detection. A detailed description of the sample preparation and analytical procedure is published elsewhere (Neuhäuser-Berthold et al., 1988a; Biotronik, 1983). All data are expressed as mean $\pm$ SEM and unpaired Student's t-test was used for statistical analysis.

Results

Weight gain and nitrogen balance

When parenteral nutrition was started, the rats had an average weight of 188 ± 1.3 g. There was no statistical significance in the initial weight between the various groups participating in the 3 experiments. Fig. 1 shows the daily weight in the course of experiments A, B, and C for the respective groups. A statistically significant difference in weight could not be observed between the respective groups in any of the experiments. There is, however, a slight tendency to increased weight gain in the groups receiving free amino acids when compared to those receiving peptides.

Similar results were recorded for the daily N-balances in the course (Fig. 2) of the respective experiments. The change from oral food consumption ad libitum to the intravenous nutritional regimen is associated with low or even negative values for weight gain and nitrogen balance. However, within 3 days weight gain and nitrogen balance reached a steady level and at that point the test period was initiated. The results for cumulative weight gain and cumulative

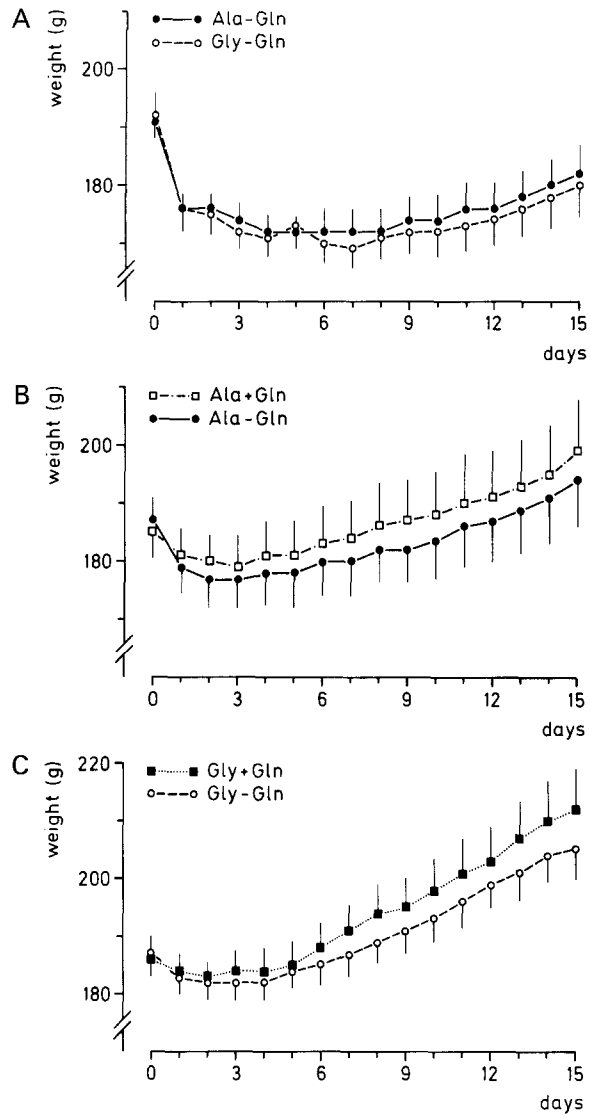

Fig. 1. Daily weight in the course of experiments **A**, **B**, and **C** (mean ± SEM)

N-balance during the test period are summarized in Table 1. Although the values for free amino acids are slightly higher when compared to the peptides, this difference is not significant in any of the experiments. When comparing the results of the 3 experiments with each other, however, a considerable deviation in cumulative weight gain and nitrogen balance can be observed.

Amino acids in plasma and urine

In Fig. 3 plasma levels of free Gly, Gln, and Ala of the various infusion groups are illustrated as mean values ± SEM. During the infusion with Ala-Gln plasma concentrations of Ala but not of Gln were significantly increased when compared to plasma levels during infusion with the corresponding free amino acids. There is no difference in the Gly concentration between these groups. Similarly, there

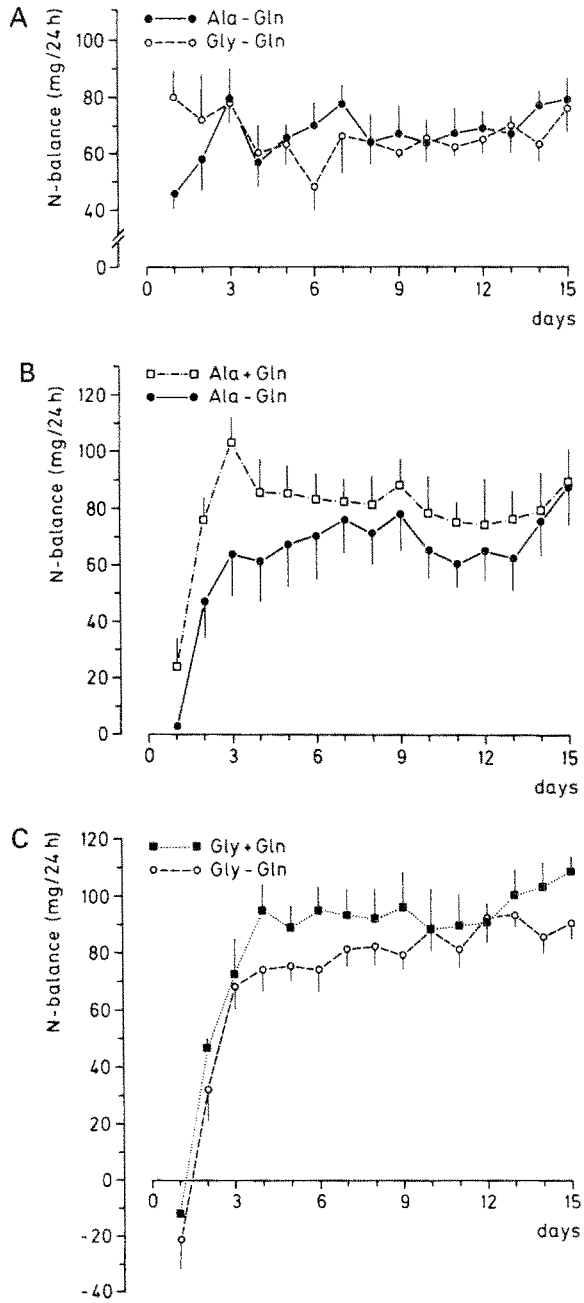

Fig. 2. Daily N-balances in the course of experiments **A**, **B**, and **C** (mean ± SEM)

is no difference in the Ala concentration in plasma for the animals infused with either Gly-Gln or corresponding free amino acids. Plasma values were, however, lower for both Gln and Gly during infusion with Gly-Gln when compared to the results following infusion with the corresponding free amino acids. Lower plasma Gln levels were observed in experiment A for the rats receiving the Gly-Gln when compared to the rats given Ala-Gln (375 ± 22 μmol/L for group II vs. 448 ± 22 μmol for group I; $p < 0.05$). The daily urinary excretion of free

Table 1. Weight gain and cumulative N-balance during the 3 experiments (mean ± SEM)

Experiment	Group	Weight gain (g)	Cumulative N-balance (mg)
A	I (Ala-Gln)	8 ± 3.2	845 ± 78
	II (Gly-Gln)	10 ± 0.6	819 ± 38
B	III (Ala-Gln)	17 ± 3.5	824 ± 132
	IV (Ala + Gln)	20 ± 3.9	943 ± 122
C	V (Gly-Gln)	23 ± 2.0	1016 ± 50
	VI (Gly + Gln)	29 ± 4.1	1131 ± 100

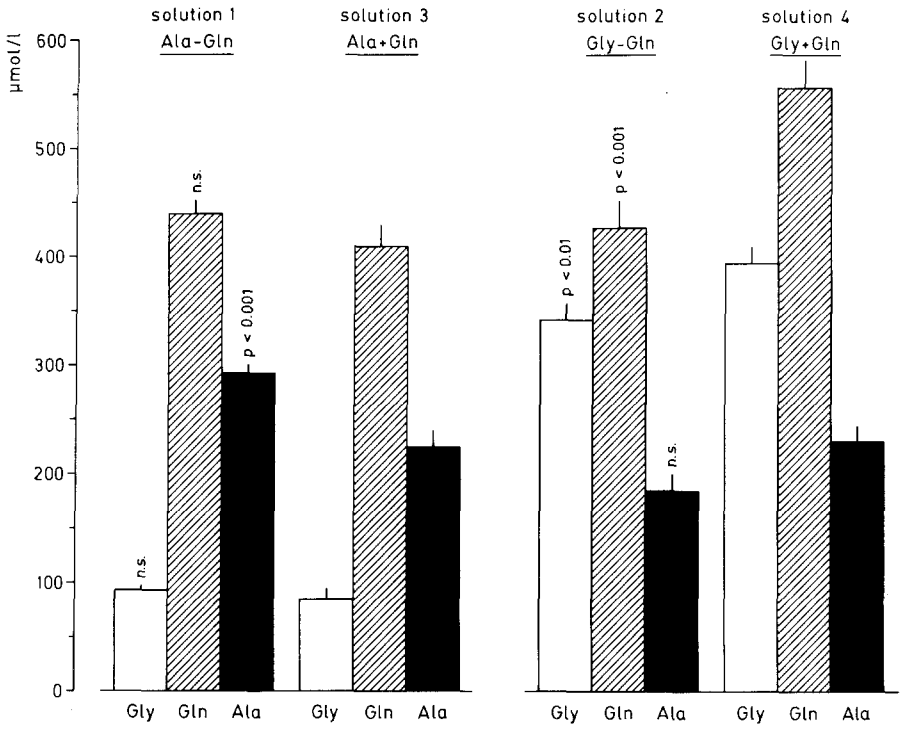

Fig. 3. Plasma levels of free glycine, glutamine and alanine during infusion with the 4 test solutions (mean ± SEM). Statistical comparisons using Student's *t* test of plasma concentrations of glycine, glutamine, and alanine between the groups infused with either alanyl-glutamine or the respective free amino acids and between the groups receiving either glycyl-glutamine or the respective free amino acids (*n.s.* not significant)

Gly, Gln and Ala in urine is summarized in Fig. 4. The amounts are very small and without quantitative importance. The increased plasma levels of Ala during the infusion with Ala-Gln are associated with an increase in the excretion of Ala. During infusion with Gly-Gln, low Gln and Gly levels are also concomitant with lower excretion in urine. Urinary losses of Gln were significantly lower in experiment A during infusion with Gly-Gln than during the infusion with Ala-Gln (0.95 ± 0.027 µmol/24h for group II vs. 2.16 ± 0.089 µmol/24h for group I; $p < 0.0001$).

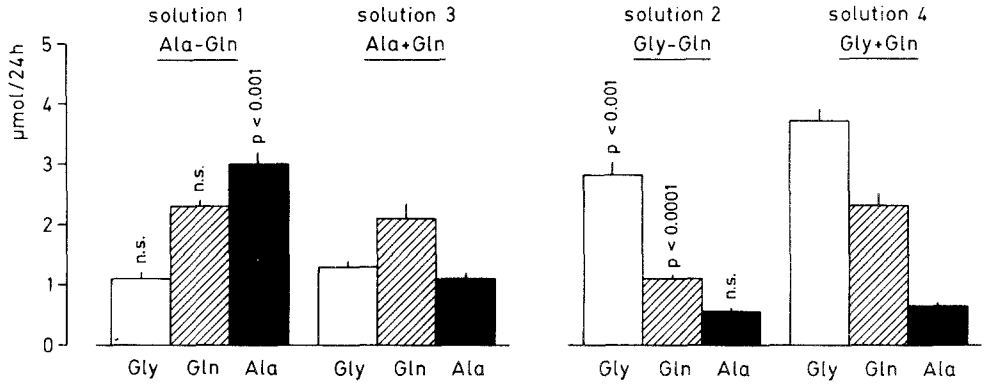

Fig. 4. Daily urinary excretion of glycine, glutamine, and alanine during the infusion with the 4 test solutions (mean ± SEM). Statistical comparisons using Student's *t* test of urinary concentrations of glycine, glutamine, and alanine between the groups infused with either alanyl-glutamine or the respective free amino acids and between the groups receiving either glycyl-glutamine or the respective free amino acids (*n.s.* not significant)

Ala-Gln and Gly-Gln in plasma and urine

Plasma levels of the two peptides and the daily urinary excretion are shown in Fig. 5. The amount of the peptides given intravenously was equal for both groups and amounted to 9.524 mmol × kg^{-1} × day^{-1}. Plasma levels were 87 ± 6.6 for

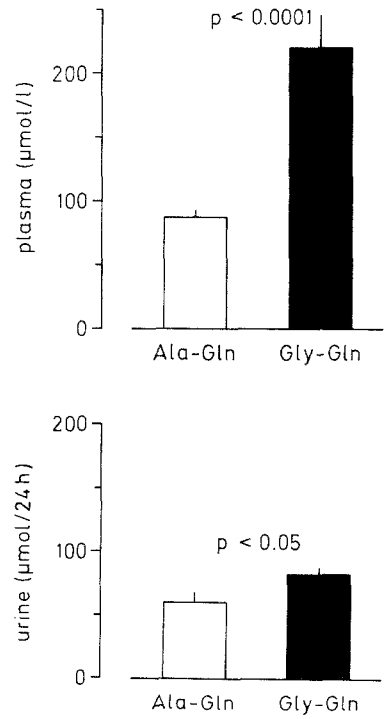

Fig. 5. Plasma levels and daily urinary excretion of alanyl-glutamine and glycyl-glutamine during infusion with the respective peptides (mean ± SEM). Statistical comparison between the respective groups was performed by using Student's *t* test

Ala-Gln and 222 ± 12.7 μmol/L for Gly-Gln. The higher plasma levels for Gly-Gln are also associated with the significantly higher urinary losses of 80.1 ± 5.4 μmol × day^{-1} for Gly-Gln and 61 ± 5.2 μmol × day^{-1} for Ala-Gln. The percent excretion rate was estimated at 3.7% for Ala-Gln and at 4.3% for Gly-Gln.

Discussion

In vivo utilization of amino acids can be studied very efficiently by N-balance and weight gain. We therefore based our experimental model for long-term parenteral nutrition in the growing rat on these classical methods. The results show, that both peptides are similarly well utilized for protein synthesis and growth and that infusion with the corresponding free AA yields only insignificantly improved results. It is questionable whether a larger number of animals within the groups or a prolonged infusion period would supply more significant results. Earlier studies showed that the model applied is sensitive enough to detect significant differences under the conditions described above (Neuhäuser-Berthold et al., 1988d; Neuhäuser et al., 1985; Neuhäuser et al., 1986).

The considerable differences observed in cumulative N-balance and weight gain are notable, when comparing the results of the 3 experiments. Experimental conditions were kept constant throughout the 3 experiments. There was no significant difference in weight between the groups participating in experiments A, B, and C at the beginning of the infusion period. However, at the beginning of the test period weight of the respective groups was slightly higher in experiment C (183 g ±2.1) and slightly lower in experiment A (173 g ± 2.0) when compared to experiment B (178 g ± 3.4). Growth rates of rats can vary considerably even within the same strain. As the 3 experiments were carried out consecutively and not simultaneously this might be the most reasonable explanation for the differences in weight gain observed for the same groups.

Plasma levels of Gly-Gln were approximately 3 times higher than those of Ala-Gln. This is in accordance with results reported by other investigators who estimated plasma half-life during bolus injections in rats and in man to be significantly longer for Gly-Gln (Fürst et al., 1987; Adibi et al., 1986; Karner et al., 1987; Brandl et al., 1987). It further appears to be the explanation for the lower concentration levels of free Gln in plasma resulting from infusion with Gly-Gln when compared to the levels observed in animals infused with either free Gly and Gln (experiment C) or Ala-Gln (experiment A). Although urinary excretion of Gln is significantly lower during the infusion with Gly-Gln, this divergence may be negligible in view of the very small excretion range in all groups. Gln losses are mainly determined by urinary excretion of the peptides which is much higher than the free amino acids. Urinary losses of Gly-Gln are significantly higher than those of Ala-Gln and may also depend on the longer plasma half-life of Gly-Gln. Nevertheless, the losses are insignificant and a comparison with other results reveals that the excretion rate during bolus injections or short term infusion studies was reported to be at a similar range by other researchers (Adibi et al., 1986; Brandl et al., 1987; Steinhardt et al., 1984). Consideration has to be given to the fact that in this study a rather high amount of peptides was infused and that the amounts required in clinical practice

will be much lower. The excretion rate might decrease when lower amounts are infused. The urinary losses of both peptides are considerably lower than those of acetyl-glutamine, which were estimated to range at around 20% in the rat (Neuhäuser-Berthold et al., 1988a) and up to 40% in man (Magnusson et al., 1987). This might justify the use of peptides despite the higher costs involved than with acetyl-L-glutamine. The question might be raised as to whether the above results recorded in the rat are also valid in man. Rapid hydrolysis is a prerequisite for the utilization of Gln from these derivates. Recent studies showed that hydrolysis capacity of various tissues for these Gln derivates in rats and in man is highly similar (Neuhäuser-Berthold et al., 1988b; Neuhäuser-Berthold et al., 1988c). We therefore assume that the peptides investigated are equally well utilized in man when supplied intravenously.

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Authors' address: Prof. M. Neuhäuser-Berthold, Fb Haushalt und Ernährung, Fachhochschule Fulda, Marquardstrasse 35, D-W-6400 Fulda, Federal Republic of Germany.

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