Comparative Value of Various Sources of Nonspecific Nitrogen for the Human

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Nonspecific nitrogen may be defined as nitrogen from any metabolically usable nontoxic source. Thus, it may include nitrogen from nonessential amino acids, from essential amino acids fed in excess of need, or from such nonamino acid, nonprotein sources as urea or diammonium citrate. Research results suggest that level and source of nonspecific nitrogen intake influence apparent adequacy of dietary protein in general and of specific essential amino acid requirements of humans.

Nonspecific nitrogen is herein defined as nitrogen from any metabolically usable, nontoxic source. Hence, it may include nitrogen from protein, essential amino acids, nonessential amino acids, or such sources as urea or diammonium citrate. The term nonessential nitrogen is sometimes used in the same sense but, by implication if not by definition, the term nonessential nitrogen implies exclusion of nitrogen from excess essential amino acids and also suggests a no-requirement function. Nonspecific nitrogen sources were used to spare essential amino acid requirements in the early, classical studies designed to determine minimal specific essential amino acid requirements. However, problems relative to the influence of both level and source of nonspecific nitrogen have not been cleared.

NONSPECIFIC NITROGEN SOURCES IN DETERMINING EAA REQUIREMENTS

In attempting to define minimal needs of essential amino acids for humans, both adults and children, investigators came to the conclusion that sources of nitrogen other than that supplied by the essential amino acids must be provided to allow for maximal synthesis of the nonessential amino acids if stated minimum values of the essential amino acids were to be truly minimum. Obviously, feeding the essential amino acids in excess of need would also fulfill this function; however, this would have led to an overestimation of essential amino acid need (Rose and Wixom, 1955).

In the classical studies of Rose and coworkers (1955a–c, 1957), Leverton (1959), Swendseid and Dunn (1956), Reynolds (1957), and Holt and Snyderman (1956) on essential amino acid requirements of humans, a variety of sources and levels of these nonessential sources of supplementary nitrogen were used. Estimation of minimal needs for the various essential amino acids varied greatly among reports of these workers. Although experimental procedures varied in respects other than source and level of nonspecific nitrogen level and source, this factor has received a considerable amount of investigation as a means of explaining variation in results of these workers.

In controlled feeding studies involving humans in which semisynthetic diets were (and are) used, the most commonly used sources of nonspecific nitrogen were glycine, diammonium citrate, glutamic acid, mixtures of these, or mixtures of nonessential amino acids in the proportion found in casein or albumin. No single research report has been published in which all of these sources have been compared; however, several attempts have been made to compare the effectiveness of selected groups of these to maintain nitrogen equilibrium in human adults.

LEVEL *VS.* SOURCE OF NONSPECIFIC NITROGEN

The comparative effectiveness of a mixture of crystalline nonessential amino acids in the pattern of whole egg or an isonitrogenous mixture of glycine and diammonium citrate was studied at two levels of total nitrogen intake (Chao, 1960; Kies, 1960). The essential amino acids were supplied in the pattern found in 20 g of egg protein. Nonspecific nitrogen either in the form of crystalline nonessential amino acids or as a mixture of glycine and diammonium citrate supplied either 4.0 or 8.0 g of nitrogen/day. At the higher total nitrogen intake level, subjects were in positive nitrogen balance. Both sources were seemingly equally effective as nonspecific nitrogen sources. However, at the 4.0 g of nitrogen intake level, mean nitrogen balance of subjects, while receiving the supplement composed of a mixture of crystalline, nonessential amino acids, was −0.89 g of N/day, but while receiving glycine and diammonium citrate the mean nitrogen balance was −1.35 g of N/day. This suggests that a mixture of nonessential amino acids is a more efficient source of nonspecific nitrogen than is a mixture of glycine and diammonium citrate under conditions of severe restriction of total nitrogen intake.

In another paired comparison type study, Swendseid *et al.* (1960) reported that a mixture of glycine and diammonium citrate was just as effective as a mixture of purified nonessential amino acids in maintenance of nitrogen equilibrium in young men fed semisynthetic diets but that glycine was significantly poorer as a source of nonspecific nitrogen than a similar mixture of nonessential amino acids. Before one concludes that glycine must therefore be a significantly poorer source of nonspecific nitrogen than diammonium citrate, it should be noted that the diammonium citrate–nonessential amino acid comparison was carried out at a total nitrogen intake level of 10.0 g of N/day, while the glycine–nonessential amino acid comparison was done at 6.5 g of N/day intake level. As seen in the Kies (1960) and Chao (1960) studies, level of nitrogen intake as well as source seemingly influence comparative effectiveness. These results suggest that when optimal amounts of nonspecific nitrogen are included, the source of nonspecific nitrogen is of little importance. However, when lower amounts of nonspecific nitrogen are fed, the source becomes more significant.

In a later study, several individual and combination sources of nonspecific nitrogen were studied in adult humans in diets supplying 9.0 g of N/day (Clark *et al.*, 1963). Of this amount the test nonspecific nitrogen sources were fed to supply 4.68 g of N/day. Under these conditions, the following nitrogen balances resulted: glycine-glutamic acid-diammonium citrate mixture, +0.51 g; glycine–glutamic acid mixture, +0.62; glycine–diammonium citrate mixture, +0.27; glutamic acid–diammonium citrate mixture, +0.34; glycine alone, +0.39; and diammonium citrate, +0.08 g of N/day, respectively. A statistically significant difference in values (5% level) was found only between the two extreme values; *e.g.*, between nitrogen balance achieved when a mixture of glycine and glutamic acid was fed and when diammonium citrate alone was used. Urinary excretion of lysine, glycine, glu-

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tamic acid, and free α-amino nitrogen of subjects receiving the various sources of nonessential nitrogen was also determined. Source of nonspecific nitrogen had no influence on excretion of lysine. When glycine was fed as the supplementary nitrogen source either singly or in a mixture, the excretion of glycine was increased. However, when glutamic acid was the source of supplementary nitrogen either singly or in combination, no increase was observed in its excretion over that seen when other sources of nonspecific nitrogen were used. Free α-amino acid nitrogen excretion was greatest when glycine was the nonspecific nitrogen source.

A crystalline mixture of nonessential amino acids in the proportion found in casein was found to be more effective in support of nitrogen equilibrium than a mixture of glycine and diammonium citrate or a mixture of glycine, diammonium citrate, and glutamic acid in studies reported by Anderson *et al.* (1969). These authors also reported variations in plasma amino acid patterns and excretion of urinary amino acids seemingly due to source of nonspecific nitrogen. Although Swendseid *et al.* (1960) and Watts *et al.* (1964) reported no differences in effectiveness of mixtures of nonessential amino acids and of glycine and diammonium, failure to demonstrate a difference does not necessarily indicate that no differences exist.

ESSENTIAL TO NONESSENTIAL AMINO ACID RATIOS

As reviewed by Linkswiler (1972), several studies have been completed by her group in which the percent of nitrogen furnished by essential amino acids was increased, while nitrogen furnished by diammonium citrate and glycine was decreased. At medium total nitrogen intake levels, as the percentage of nitrogen furnished by the essential amino acids was increased, the nitrogen retention of adult subjects also increased. This suggests that possibly essential amino acid mixtures are more effective sources of nonspecific nitrogen than is a mixture of glycine and diammonium citrate, or that essential amino acids *per se* facilitate protein synthesis. This is not particularly surprising since several studies have shown that nonessential amino acid mixtures are more effective in supporting nitrogen retention than mixtures of glycine and diammonium citrate.

UREA AS A SOURCE OF NONSPECIFIC NITROGEN IN NORMAL HUMANS

It is rather strange that urea has not usually been included in these comparative studies of chemically defined nonspecific nitrogen sources. This is unfortunate since urea was frequently used as a source of nonspecific nitrogen by Rose (1957) and Holt and Snyderman (1956) in the establishment of amino acid requirements of adult men and infants. The general objective of two studies from our laboratory was to compare the effectiveness of urea and diammonium citrate as sources of nonspecific nitrogen (Kies and Fox, 1973).

The first study consisted of four experimental parts, each composed of a 5-day nitrogen adjustment period and two experimental periods of 5 days each. During one experimental period an isonitrogenous mixture of glycine and diammonium citrate was the source of nonspecific nitrogen and, during the other, the source of nonspecific nitrogen was an isonitrogenous mixture of urea and diammonium citrate. During the four parts, these nitrogen sources were added to the diets to supply 1.77, 4.77, 7.77, and 10.77 g of N/day. The essential amino acids were fed as defined by the Rose Minimum Requirement Pattern. Accounting for all sources of nitrogen, nitrogen intake ranged from 3 to 6 to 9 to 12 g of N/day.

Mean nitrogen balance of subjects receiving urea and glycine at the 3.0 g of N level was −1.65, a value significantly lower ($p < 0.05$) than the mean value of −1.12 g of N, while receiving diammonium citrate and glycine. At the 6.0 g of N level, the comparable values were −0.85 and −0.52 ($p < 0.05$), at the 9.0 g of N level, the values were +0.12 and +0.28 (no significant difference), and at the 12 g of N intake level, the values were +0.74 and +0.72 g of N/day (no significant difference), respectively. Thus, as the level of nonspecific nitrogen supplementation was increased, the nitrogen retention of subjects also increased and the difference in effectiveness between the two test sources of nonspecific nitrogen was decreased.

The second study of this series was composed as a 2-day depletion period, a 3-day adjustment period, and two experimental periods of 5 days each. During the two experimental periods, diammonium citrate or urea was fed as a single source of nonspecific nitrogen at a 4.3 g of nonspecific N intake level (6.0 g total N intake). Other aspects of the study were essentially the same as in the one just described. Mean nitrogen balance of subjects receiving the urea supplement was −0.42 g of N and, while receiving diammonium citrate, was −0.24 g of N ($p < 0.05\%$). These results clearly suggest that urea is a less efficient source of nonspecific nitrogen than diammonium citrate.

NONSPECIFIC NITROGEN AND CLINICAL NUTRITION

Urea utilization by humans has been studied in a somewhat different manner in relationship to dietary treatment of uremic patients. While data obtained from uremic patients does not transfer well to normal, healthy human beings, some insight can be obtained from these studies on human utilization of nonspecific nitrogen. High levels of blood urea nitrogen found in patients with uremia can seemingly be reduced if small amounts of essential amino acids in idealized patterns are included in protein-free diets or in intravenous feedings as reported by Giordano (1963), Giordano *et al.* (1968) and Giovannetti and Maggiore (1964). In addition to reducing blood urea, nitrogen balances of patients are improved. Using both normal and uremic patients, N_{15} from urea was found in nonessential amino acids of blood albumin. Administration of an antibiotic to reduce intestinal microorganism populations and urease activity resulted in a decrease in urea utilization. In a sense, this therapeutic approach can be thought of as making use of endogenous urea as a source of nonspecific nitrogen.

Human utilization of urea nitrogen in low energy diets was investigated by Gallina and Dominguez (1971). These authors concluded that in low calorie diets, urea can replace part of the high biological value protein component of the diet with no decrease in nitrogen retention if a sufficient amount of glucose is also provided for synthesis of nonessential amino acids. Thus, variation in results of various studies on comparative effectiveness of various sources of nonspecific nitrogen may actually be related to dissimilarities in other parts of the experimental diets.

The previous discussion indicates that nitrogen balances of subjects maintained on assumed adequate intakes of essential amino acids in relatively simple, chemically defined diets can be rather drastically changed by source–level variations in dietary supplements of nonspecific nitrogen.

The possibility exists that at least some of these apparent changes are just that, apparent rather than real. It may be that these changes are the result of inherent errors in the nitrogen balance method. This could very well be true. However, nearly all information on amino acid requirements of humans is based on nitrogen balances of subjects maintained on experimental diets containing different levels and sources of nonspecific nitrogen. For example, urea alone or in combination with glycine was used as the nonspecific nitrogen source supplement to obtain minimal essential amino acid need values in the studies of Rose and coworkers. However, individual papers do not state whether glycine plus urea or urea alone were used for establishment of quantitative needs of each amino acid (Rose *et al.*, 1954, 1955b,c,d). Total nitrogen intake in these studies ranged from 7.0 to 10.0 g of N/subject/day. Glycine plus diammonium citrate or glycine alone was used in the studies of Leverton and coworkers with total dietary nitrogen in different studies ranging from 6.2 to 9.5 g of N/subject/day (Leverton *et al.*, 1956a,b,c,d). Thus, inconsistency in level and source of nonspecific nitrogen use is found in reports from the same research groups as well as among research groups. Depending upon whether an efficient source was used and depending upon level of nonessential nitrogen fed, the requirements of essential amino acids now accepted as standards are either over- or underestimated.

NONSPECIFIC NITROGEN AND CEREAL-PLANT PROTEIN RESOURCES FOR HUMANS

Another way of looking at comparative effectiveness of various sources of nonspecific nitrogen is in relationship to food sources. In our laboratory we have been very interested in factors determining adequacy of cereal and plant resources to meet the protein needs of humans.

In one of our early studies (Kies *et al.*, 1965a), white degerminated corn meal was fed to provide 4.0, 6.0, or 8.0 g of N/day. As the level of corn nitrogen in the diet was increased, the level of nitrogen retention of subjects was also increased. Subjects in general were in positive nitrogen balance at the 8.0 g of N level but were in negative balance at the 6.0 g of N level. To determine why the 8.0 g of corn N intake level was adequate and the 6.0 g of N intake level was inadequate, another study was run. Corn was again fed to provide 6.0 g of N to which individual supplements of essential amino acids singly or in combination were added in amounts found in 2.0 g of N from corn. All diets were equalized to the 8.0 g of N intake level with glycine and diammonium citrate. Glycine and diammonium citrate in one period provided a supplement of 2.0 g of N. All the supplements were equally effective in the reestablishment of positive nitrogen balance. Using a somewhat similar approach. Snyderman *et al.* (1962) and Scrimshaw *et al.* (1966, 1968) found that milk and egg protein could similarly be diluted with nonspecific nitrogen with no adverse effect on nitrogen balance. It should be remembered that egg and milk are very high quality protein resources in comparison with ordinary corn.

In a rather long series of studies, other aspects of nonspecific nitrogen supplementation of corn and other plant protein products were undertaken in our laboratory. This work was recently reviewed in a previous paper (Kies, 1972a). The effect of quality and level of nonspecific nitrogen supplementation of corn diets fed at the 6.0 g of N level, an inadequate amount to provide for nitrogen equilibrium in human adults, was studied (Kies *et al.*, 1967b). Supplements added 2 or 6 g of N/day from milk, gelatin, soybean meal, zein, or a mixture of glycine and diammonium citrate. All sources of nonspecific nitrogen resulted in improvement in nitrogen retention with increased level of nonspecific nitrogen intake. However, milk and soybean meal were superior to other sources, suggesting that the concept of importance of nonspecific nitrogen intake does not eliminate the concept of importance of protein quality. Zein was somewhat superior to glycine and diammonium citrate. However, since glycine and diammonium citrate supplementation did result in improvement in nitrogen retention, some of the greater improvement shown by the milk supplement must be credited to the effect of nonspecific nitrogen and not solely to that of improvement in essential amino acid intake.

Results of other studies of this series suggest that lysine requirements of humans as provided by corn are lower with the presence of high level nitrogen supplementation, that nonspecific nitrogen may spare protein need, that order of limiting amino acids may be affected by level of nonspecific nitrogen intake, and that positive nitrogen balances achieved with nonspecific nitrogen supplementation are relatively permanent rather than being of a transitory adjustment nature (Kies and Fox, 1970, 1972a,b, 1973; Kies *et al.*, 1965a,b, 1967a,b).

Nonspecific nitrogen supplementation of suboptimal cereal diets does not always result in improvement in protein nutritional value. Clark *et al.* (1971), working with rice diets, failed to show any improvement with a supplement of glycine, diammonium citrate, and glutamic acid. However, subjects did show better nitrogen retention when fed diets containing a higher level of total protein—another way of saying nonspecific nitrogen.

That nonspecific nitrogen level and source seemingly influence both total protein and essential amino acid requirements of humans does have important practical implications in another aspect. This is in relationship to the relative importance of protein quantity and protein quality in diets and in basic foods making up these diets. There is some evidence to suggest that essential amino acid requirements are changed when total dietary nitrogen is altered. More specifically, lysine requirement is seemingly lower when total dietary nitrogen is high.

The discovery of the higher lysine content of opaque-2 corn has generated tremendous interest and awareness to the possibilities of improvement in protein quality of cereal and plant products *via* genetic alteration to increase levels of first-limiting amino acids in these products (Mertz and Nelson, 1966). The importance of this discovery cannot and should not be underestimated. However, perhaps there have been some unfortunate side effects. One of these might be called the development of the "lysine cult." This refers to the idea that an increase in lysine content per gram of protein is the only way in which the value of a cereal as a source of protein can be achieved.

There is a tendency, at least at the extreme ends of the scale, for lysine content/g of protein of wheat grain to decrease as the total protein of the grain increases; the stress on lysine content/g of protein thus may encourage the proliferated use of low protein wheats in the interest of "good nutrition." Humans consume wheat and other cereals in terms of quantities of grain (as flour contained in bread, etc.) rather than in terms of quantities of protein. Encouragement of the development of higher protein cereals and direct use of them by human populations may offer a double advantage. First of all, a higher protein cereal with a somewhat low lysine (or other first-limiting amino acid)/g of protein basis may actually provide more lysine (or other first-limiting amino acid) on a per unit grain basis than does a low protein cereal having a relatively high lysine content (or other first-limiting amino acid) on a per gram of protein basis. Secondly, a cereal having a high protein content will provide a diet having a higher nonspecific nitrogen content than will a cereal having a low protein content, assuming that both cereals are consumed in equal quantities. Under these conditions, the higher nonspecific nitrogen intake may result in a lowering of quantitative requirement of the first-limiting amino acid.

This does not eliminate ideas related to protein quality but does suggest that both must be taken into consideration. How then can one answer a question such as, "Should wheat breeders aim for increased lysine or increased protein in wheat grain to give improvement in this product as a source of protein for humans?" On the basis of our work with mouse growth and limited work with humans, one cannot predict nutritional value with certainty on the basis of chemical analyses for either total protein content or total lysine content per gram of cereal or per gram of grain (Kies, 1972b). When compared on the basis of equal quantities of grain, a low protein wheat having a higher lysine content generally (but not always) gives "better" results than a low protein wheat with a low lysine content. However, when one compares a high protein wheat with a relatively moderate lysine or low lysine content against a low protein wheat with a high lysine content, the results are not predictable.

It would be very nice to be able to tell how well a product will do in feeding trials on the basis of chemical analysis; however, in a sense this situation is highly encouraging because it offers alternatives to improvement in protein nutrition of world populations.

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