points of 104.9, 146.7, and 178.1, assuming that at 100 the cocks have been fully repleted to their starting status before N depletion began, the remainder appears to be equivalent to the biological values for the three N sources. In the case of aspartic acid this value was essentially zero, a not unexpected finding, since for tissue protein synthesis all the essential amino acids must be provided. In the case of gelatin the value (46.7) is not too different from the biological value that has been reported for the chick (Summers and Fisher, 1962) for this protein. Finally, the value of 78 for fish meal is also in line with the biological value of fish meal as obtained with the growing chick (Summers and Fisher, 1962).

Although these studies shed little light on the nature of the retained N, they do indicate that nonessential N can and does play an important role in intermediary N metabolism in the body. As a follow-up to the investigation just cited, additional adult cocks were N-depleted on a protein-free diet (Ashley and Fisher, 1967). One group was fed a maintenance mixture of essential amino acids only, which constitutes an inadequate amount of total N; a second group was given the same diet but with additional N in the form of aspartic acid to meet not only the maintenance needs for essential amino acids but also the total N needs to permit repletion. Table III shows the pertinent body weight and N loss measurements. Those cocks receiving the essential amino acids but an inadequate amount of total N were in severe negative N balance, whereas those receiving the additional aspartic acid N were in approximate N equilibrium. Table IV shows certain muscle N constituents, including RNA and transaminase enzyme concentrations. While the feeding of the essential amino acids alone repleted the RNA, GOT and GPT concentration in muscle, the further addition of aspartic acid N to the essential amino acid mixture produced additional increases in RNA and both transaminase enzymes. This provides additional evidence for the direct utilization of nonessential N perhaps as a kind of reserve pool that may act as a buffer during periods of deprivation or inadequate protein intake. It may well be that the role played by such N sources is one of enzyme induction which may stimulate or decrease protein anabolism as well as catabolism, depending upon the dietary N intake.

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Nonessential Nitrogen Utilization by Children and Adolescents

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Demands for growth may influence the quantity and quality of dietary protein needs. Thus, children and adolescents should be given special consideration in the discussion of utilization of nonessential nitrogen by human beings. Studies have shown, in general, that nonessential nitrogen can contribute significantly to the protein needs of children and adolescents. Nonspecific nitrogen was considered the first limiting nitrogenous factor in milk for human infants by Snyderman *et al.* (1962). In another laboratory, preadolescent girls responded as well to a supplement of nonessential nitrogen as to a mixture of essential amino acids considered the most limiting in a diet simulating that of low-income southern families. Urea supplementation improved nitrogen retention in adolescent boys when opaque-2 corn supplied nearly the sole source of dietary protein. That nonessential nitrogen can be effectively utilized by children to meet protein needs of growth must be considered a tentative conclusion because of the dearth of studies on children. A better understanding of this aspect of human utilization of nonspecific nitrogen is needed if nonspecific nitrogen is to be considered as a potential means of expanding protein resources for human consumption.

Protein nutrition in young children is considered the major nutritional problem of the world by a joint FAO/WHO committee (1965). Protein demands during growth may be more stringent than for adult maintenance. Thus, study of human protein utilization is incomplete without including young subjects from infancy through adolescence. Since chemical and genetic alterations of food materials are considered possible methods of improving the world's protein supply, there is a critical need for nutrition scientists to define human protein requirements as precisely as possible. More definitive information on human protein requirements throughout the life cycle is also needed for use in nutrition education programs. The role of nonspecific nitrogen in meeting human protein needs is not clear. Most of the work in this area has been

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done using human adults as subjects. Although few in number, studies providing insights into the role of nonessential nitrogen in the nutriture of children and adolescents will be discussed.

Much of what is known about the utilization of nonspecific nitrogen has come from amino acid requirement studies. Rose and coworkers, for example, in his classic studies (1955) using adult men, fed liberal quantities of glycine or urea in combination with mixtures of essential amino acids as sources of dietary nitrogen. The glycine and urea were provided as a source of nitrogen for the synthesis of nonessential amino acids. The effect of kind and amount of nonessential nitrogen was not evaluated. In most studies the nitrogen level of the diet has been maintained at 9 or 10 g daily, an amount which compares with nitrogen from a 50- to 60-g protein intake. Likewise, when the essential amino acid requirements of children were studied (Nakagawa *et al.*, 1960, 1961a,b, 1962, 1963, 1965) it was recognized that a source of unessential nitrogen was necessary in order to meet the total protein requirement and to adequately assess the minimum amount of each amino acid required. Nakagawa and coworkers (1960) reported that 10 to 14-year-old boys maintained a positive nitrogen balance at levels of 11 or 12 g of total nitrogen daily. This level was arrived at by a series of manipulations both in quantity of essential amino acid mixture and in the supplementary sources of nitrogen. They demonstrated that either essential or nonessential nitrogen could be a factor limiting the attainment of positive nitrogen balance in adolescent boys. The results of their studies indicated that amino acid requirements of adolescent boys were indeed higher than those for men, approximately double, except for tryptophan which was similar to the adult male requirement. Girls studied by the same group (Nakagawa *et al.*, 1965) maintained positive nitrogen balance on the minimum essential amino acid pattern determined for boys. One difference was that girls demonstrated greater variability in the total amount of nitrogen required, which the authors attributed to the earlier growth spurt. Presumably girls with the higher requirements were in a more rapid phase of the growth spurt. The demonstration that total nitrogen intake was an important factor in determining amino acid needs during growth emphasizes the importance of nitrogen other than that from essential amino acids in meeting protein needs.

Some important insights into the role of nonessential nitrogen in meeting protein needs of infants have come from the work of Snyderman and her associates (Pratt *et al.*, 1955; Snyderman *et al.*, 1962). The essential amino acid requirements of infants were determined by this group (Pratt *et al.*, 1955) by feeding a synthetic amino acid mixture simulating the essential and nonessential amino acid pattern of human milk. This approach differed from that used by Rose or other investigators in that the requirements for essential amino acids were determined in the presence of amounts of nonessential amino acids found in natural protein. This amount was much less than that of nonspecific nitrogen supplied in the experimental diets used in the determination of minimum essential amino acid requirements of adults. The results suggest that the amino acid requirements of adults, because of the experimental diet used, are closer to minimal requirements than the amino acid requirements of infants. However, it also emphasizes that the adult requirements, because they were determined under artificial conditions using large amounts of synthetic unessential nitrogen sources, may be less applicable to conditions of ordinary human feeding than the requirement of infants using a diet more closely related to a diet containing natural foods.

To determine the first limiting nitrogenous factor for man in natural protein, Snyderman *et al.* (1962) studied the utilization of milk protein in infants. Protein intake was reduced stepwise, keeping calories constant, until weight gain was arrested and nitrogen retention was subnormal. Normal weight gain and nitrogen retention were restored with the addition of unessential nitrogen from either glycine or urea to the diet. Unessential nitrogen was considered the first limiting nitrogenous factor in milk to meet the needs of human infants. By implication, unessential nitrogen may be the first limiting factor for other food sources of intact proteins. Snyderman also demonstrated that unessential nitrogen supplements contributed to protein synthesis by feeding [^{15}N]urea in one case and [^{15}N]ammonium chloride in another. Substantial incorporation of labeled nitrogen into plasma protein and hemoglobin of infants resulted from feeding either source of unessential nitrogen.

Even in studies where primary attention has been given to improvement of diets by additions of specific amino acids, some recognition has been given to the role of other sources of nitrogen. Thus, Bressani *et al.* (1963) suggested that reference amino acid patterns for young children should specify not only the upper and lower limits for the amount of each amino acid but also the upper and lower limits of protein intake over which the pattern applies. This in effect recognizes that total protein intake influences the need for specific amino acids. Presumably with diets of higher protein nitrogen content the need for essential amino acids would be decreased. It also suggests an interrelationship between nonessential and essential sources of nitrogen.

Studies which have attempted to establish minimum protein requirements of 7–9-year-old girls using protein from mixtures of natural foods have suggested interrelationships between essential and nonessential nitrogen. While diets providing as little as 18 to 20 g of protein per day from certain combinations of foods supported a slightly positive balance of 0.3 g per day, a diet providing 25 g of protein and patterned after the typical southern low-income diet proved inadequate for this purpose (Abernathy *et al.*, 1970). In an attempt to explain this inconsistency, another study (Abernathy *et al.*, 1971) tested the same diet supplemented with either a mixture of lysine, methionine, and threonine in amounts to make the protein score of this protein source equivalent to 80% of egg values or with an isonitrogenous amount of ammonium citrate. Nitrogen balances resulting from these treatments did not differ significantly. Both groups retained approximately 0.5 g of nitrogen per day. The results suggested that nitrogen itself was as effective as specific amino acids in improving the protein nutriture of these subjects. The results imply that the value of nonspecific nitrogen supplementation applies not only to highly artificial dietary regimens employed in laboratory situations but may also be extended to ordinary mixed diet situations.

The value of nonspecific nitrogen supplementation of diets has been extensively studied at the University of Nebraska Food and Nutrition laboratories using adult human subjects (Kies and Fox, 1970; Kies *et al.*, 1965a,b, 1967a,b). In these studies nitrogen from any nontoxic metabolically utilizable source has been shown to improve nitrogen balance response when diets are limited in total nitrogen content. Since protein needs for growth are generally believed to be more critical than those for maintenance, it seemed worthwhile to investigate the effect of nonspecific nitrogen supplementation of diets for growing subjects. Adolescent boys were selected for study because they experience rapid growth and also because they are old enough to understand research procedures and the responsibilities and hazards involved in human experimentation.

Seven adolescent boys 12 to 16 years of age participated in a 23-day metabolism experiment in which almost all of the dietary protein was provided from opaque-2 corn, a high lysine genetic strain, or from opaque-2 corn supplemented with urea (Korslund *et al.*, 1972). Corn was fed at

Table I. Experimental Plan

Period	No. of days	N from corn, g N/day	Supplement Kind	Supplement Amt, g N/day	N basal diet, g N/day	Total N, g N/day
Adj	3	6.00	None	0	0.66	6.66
Exp I	10	6.00	None	0	0.66	6.66
Exp II	10	6.00	None	4.00	0.66	10.66

Table II. Nitrogen Balances of Adolescent Boys Fed Opaque-2 Corn with or without Urea Supplementation

	Nitrogen balance, g/day	
Subject no.	6 g of N from opaque-2 corn	6 g of N from opaque-2 corn + 4 g of N from urea
390	+1.92	+2.81
392	+0.89	+2.39
393	−0.26	+1.17
397	−0.74	−0.16
398	+1.30	+2.11
399	+1.18	+1.24
402	+0.14	+0.04
Mean N balance	+0.63	+1.37

a level of 6 g of nitrogen per day, an amount expected to be somewhat inadequate for optimal protein nutrition in this age group. The urea supplied an additional 4 g of nitrogen per day. The study consisted of a 3-day adjustment period and two 10-day experimental periods. The experimental design is shown in Table I. In order not to prejudice the results, subjects received the experimental diets in random order, half receiving corn alone first and the other half corn plus urea. Nitrogen balance was used to assess response. The nitrogen balance technique compares total nitrogen excretion from urine and feces with the total nitrogen intake from foods. It is assumed that if nitrogen intake exceeds nitrogen excretion, food nitrogen is being retained in the body for the synthesis of body protein. On the other hand, if nitrogen excretion exceeds intake, it is assumed that nitrogen from food is inadequate to meet needs and that body protein is being used for this purpose. A state of nitrogen equilibrium where nitrogen excretion equals intake is usually considered the normal state for adults, while positive nitrogen balance defines adequate protein nutritional status for growing children and adolescents. Errors inherent in the nitrogen balance technique lead to "false positive" balance values. Therefore, it is important to observe the change in nitrogen retained on different experimental treatments rather than to place undue confidence in the exact amount of nitrogen retained.

In the present study the mean nitrogen balance attained on the 6 g of nitrogen intake from corn was +0.63 g/day and on a 10-g total nitrogen intake from corn plus urea supplement was +1.37 g/day (Table II). The improvement in nitrogen balance brought about by urea supplementation was statistically significant. Also, since all but one boy retained more nitrogen on the supplemented than on the unsupplemented diet, the findings seem biologically significant as well. The results were similar to those found for adult subjects (Kies and Fox, 1972).

The importance of this study was not that urea was the source of nonspecific nitrogen used nor that opaque-2 corn may be utilized as a protein source for adolescent boys. Instead, the important finding was that a nonspecific nitrogen product was effective in improving the apparent nutritional value of an intact food source of protein for growing subjects. These results support the work of Snyderman and coworkers (1962), but the improvement in protein nutritional quality of corn, even the genetically improved strain, through nonspecific nitrogen supplementation is more surprising than the similar improvement of limiting quantities of milk for infants. Protein provided by milk has a more favorably balanced essential amino acid pattern and a higher proportion of total nitrogen supplied by essential amino acids than does corn. One implication is that inexpensive sources of nonspecific nitrogen may be useful in expanding the world's protein supply and thus help to relieve the critical need for protein in the diets of all humans, including children. It must be noted, however, that long-term effects of feeding various sources of nonspecific nitrogen are not known.

Some tentative conclusions and many questions arise from studies reviewed. Apparently total nitrogen as well as essential amino acid requirements are important considerations in protein nutriture of children and adolescents just as for adults. However, requirements for essential amino acids and for nonessential nitrogen may be different for growth than for adult maintenance. The extent of these differences is not known. Demands for essential and nonessential dietary nitrogen may vary at different stages of growth since human growth proceeds at an uneven rate from infancy through adolescence. While initial study of the role of nonspecific nitrogen in the nutriture of human beings should continue to be done using adults as biological models, the unique requirements for growth cannot be determined without further study of growing humans.

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