URINARY EXCRETION OF NITROGEN FROM
\(^{15}\)N-LABELLED AMINO ACIDS IN THE MALNOURISHED
AND RECOVERED CHILD
I. GLYCINE AND LYSINE

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SUMMARY

1. Glycine and lysine with the \(\alpha\)-amino nitrogen labelled with \(^{15}\)N were fed to
marasmic and recovered infants. The excretion of total \(^{15}\)N and labelled urea in the
urine was followed for 72 h.

2. Excretion of \(^{15}\)N was lower in marasmic than in recovered infants for both
amino acids. This was mainly due to a smaller amount of \([^{15}\text{N}]\text{urea}\) excreted by the
marasmic infants.

3. \([^{15}\text{N}]\text{Lysine}\) was retained to a greater extent than \([^{15}\text{N}]\text{glycine}\) in marasmus
and after recovery. This difference was more marked in marasmus. \(^{15}\)N in urea
excreted by marasmic subjects after feeding with \([^{15}\text{N}]\text{lysine}\) was less than 5% of the
total isotope administered.

4. The results suggest that requirements of marasmic infants for individual amino
acids may differ considerably from those of the healthy infant.

Studies with \([^{15}\text{N}]\text{ammonium chloride}\) and \([^{15}\text{N}]\text{urea}\) show that children on a protein-
restricted diet incorporate much of the labelled nitrogen into protein (Snyderman, Holt,
Dancis, Roitman, Boyer & Balis, 1962). The malnourished child receiving an adequate diet
conserves nitrogen derived from orally administered \(^{15}\)N-labelled ammonium citrate (Read,
McLaren, Tchalian & Nassar, 1969). This nitrogen may be used in the synthesis of unes-
sential amino acids and may enter into transamination reactions. With lysine (Weissman &
Schoenheimer, 1941) and probably threonine (Meltzer & Sprinson, 1952) transamination
does not occur, deamination of lysine being an irreversible reaction. Protein deprivation
has little effect on the free lysine concentration in serum, liver or muscle in the rat (Waterlow
& Stephen, 1968), and in plasma in acute kwashiorkor and marasmus (Arroyave, Wilson,

In the present study the rates of nitrogen loss from \([^{15}\text{N}]\text{lysine}\) and \([^{15}\text{N}]\text{glycine}\) were mea-
sured in marasmic and recovered infants. The amino acids, labelled in their \(\alpha\)-amino nitrogen,
were administered by mouth, and the rate of excretion of the label in urine, both as total and as urea nitrogen, was measured.

MATERIALS AND METHODS

Subjects

The subjects, male Arab children, were studied while suffering from acute marasmus, as defined by a simple scoring system (McLaren, Pellett & Read, 1967), and after recovery, i.e. when standard weight for length had been achieved. \([^{15}\text{N}]\text{Glycine}\) was given to three infants with acute marasmus and three that had recovered. The same procedure was followed with \([^{15}\text{N}]\text{Lysine}\). All children were less than 10 months of age when accepted for study and about 4 months older when recovered.

Studies were commenced after the subjects had been rehydrated, electrolyte balance restored and, where necessary, infection controlled. Until they were 6 months old the diet consisted of cow’s milk formula. Thereafter a vegetable food mixture, Laubina (McLaren, Asfour, Cowan, Pellett & Tannous, 1967), was added to the diet. These diets were fed \textit{ad libitum}. All the patients made a good recovery and were discharged well.

Informed consent was obtained from the parents of each child for each experiment. The research was approved in its ethical aspects by the Research Committee of the Faculties of Medical Sciences, American University of Beirut.

Isotope administration and sample collection

The subjects were nursed on a metabolic bed throughout the experimental period. \([^{15}\text{N}]\text{-Glycine}\) with 95 atoms \% of the heavy isotope was obtained commercially from Isomet Corporation, Palisades Park, N.J., U.S.A. \([^{15}\text{N}]\text{Lysine monohydrochloride}\) with 30 atoms \% \(^{15}\text{N}\) in the \(\alpha\)-nitrogen was prepared as described by Arnstein, Hunter, Muir & Neuberger (1952). It had m.p. 263°C, and \([\alpha]_{20}+14.8^\circ\) (c=2.4 in 0.6 M-HCl). Ion-exchange chromatography showed that it contained no other amino acid or \(\text{NH}_3\).

The amino acids were dissolved in water and given by stomach tube at a dosage of 0.5 mg of \(^{15}\text{N}\) /kg body wt., followed by the first meal of the day which contained a carmine marker. A record was kept of all food taken during the following 72 h. The first meal after the test period contained a second carmine marker.

Urine was collected 3, 6, 9, 12, 24, 48 and 72 h after the administration of the test acid. Stool was collected and pooled from the appearance of the first marker, until the appearance of the second. All samples were frozen and stored at \(-10^\circ\).

Analysis of samples

All samples were prepared and analysed for total nitrogen and urea and for total \(^{15}\text{N}\) and \([^{15}\text{N}]\text{urea}\) by methods described by Read \textit{et al.} (1969). Food nitrogen intake was determined by Kjeldahl analysis. The calorific value of the dietary intake was calculated from food tables.

RESULTS

The results obtained are shown in Table 1. Both glycine and lysine were well absorbed, although the apparent absorption of food nitrogen varied considerably. As found by Read \textit{et
TABLE 1. Results of $^{15}$N glycine and $^{15}$N lysine studies in marasmus and after recovery

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* Expressed as percentage in relation to Boston standard.
al. (1969), urinary nitrogen loss as urea was consistently lower in acute marasmus than in recovery. Urinary loss of $^{15}$N from lysine was lower than that from glycine and to a much greater extent in marasmus than after recovery, as can be seen by differences in $[^{15}$N]urea excretion (Table 1). In marasmus less than 5% of lysine $^{15}$N appeared as urea, whereas much more (13–37%) of glycine $^{15}$N was excreted in this form. After recovery a higher percentage (24–35%) of lysine $^{15}$N appeared as urea than in marasmus. $^{15}$N from glycine excreted as urea after recovery was higher still (36–55%).

With glycine, in both marasmus and after recovery, the percentage of the $^{15}$N excreted in the form of urea was similar to the percentage of total nitrogen excreted as urea. However, in the case of lysine, a much lower percentage appeared in urea, especially in marasmus.

Fig. 1 shows the rate of appearance in the urine of $[^{15}$N]urea after the administration of labelled glycine in marasmus (○), and recovery (■).

**DISCUSSION**

In all the marasmic subjects urinary excretion of nitrogen originating from absorbed glycine (19–47%) was considerably greater than that coming from absorbed lysine (9–14%). There
was a similar, though smaller, difference in recovered patients (37–65% and 33–54% respectively). The differences are more striking when the urea fraction only is considered. In marasmus less than 5% of the $^{15}$N given as lysine had been excreted as urea by the end of 72 h. In recovery this value was 24–31%. In a similar period $^{15}$N from glycine excreted as urea in marasmus was 14–37% whereas in recovery it was 36–55%.

These results clearly indicate considerable differences in the extent of deamination of these amino acids. This is consistent with the results of Penn, Mandeles & Anker (1957) in their study of the kinetics of turnover of serum albumin in the rat. Of the amino acids used lysine was retained to the greatest extent and glycine to the least. The similarity of $^{15}$N and unlabelled nitrogen excreted as urea when glycine was given agrees with the suggestion (Picou & Taylor-Roberts, 1969) that glycine turnover is typical of total protein turnover in the body. The lower degree of nitrogen loss from glycine in marasmus as compared with recovery could, at least in part, be due to the additional use of amino-nitrogen known to occur in the malnourished child (Read et al., 1969).

The high retention of $^{15}$N administered as lysine in marasmus as compared with recovery suggests that a change in metabolic processes has taken place. Much of the decreased excretion is accounted for by the small loss in the form of urea, indicating that deamination of lysine is minimal. The degradation pathway leading through pipecolic acid to amino adipic acid that involves deamination as a first step, can only be operating therefore to a limited extent.

In both marasmus and recovery nitrogen loss from lysine, unlike that from glycine, occurs to a far smaller extent in the form of urea than it does in total urinary nitrogen. The behaviour of lysine is therefore not representative of total amino acid turnover in the body. The retention of almost all of the administered $[^{15}N]$lysine in marasmus suggests that in this condition the

![Graph](attachment:image.png)

**Fig. 2.** Urinary excretion of $^{15}$N in the form of urea after the administration of $[^{15}N]$lysine in marasmus (○), and recovery (●).
body’s requirement for this amino acid is raised and it may not be fully met by the diet. It is difficult to explain this apparently high requirement for lysine, since, unlike many of the other amino acids, its only known use in the mammalian body is for the synthesis of protein.

The results suggest that the requirements of the acutely marasmic child for individual amino acids may be different from those of the normal child. This could be due to differences in the various phases of recovery in the amino acid composition of the tissues laid down during ‘catch up’ growth. The almost complete retention of lysine in acute marasmus demonstrates that this amino acid is in high demand.

Unessential nitrogen from glycine is excreted to a smaller extent than normal in marasmus, as was shown to be the case for nitrogen from ammonia and urea (Read et al., 1969). However, this excretion although decreased is about three times greater than that occurring after administration of lysine. This suggests that more unessential nitrogen is available than can be utilized immediately. On the other hand, unessential nitrogen was shown to be a limiting factor in infants restricted in protein intake (Snyderman et al., 1962).

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