¹⁵N studies of endogenous faecal nitrogen in infants

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SUMMARY ¹⁵N from labelled yeast protein, fed to marasmic and recovered infants, appeared in the stool shortly after administration and continued to be excreted for 100 hours. Unabsorbed dietary ¹⁵N, which appeared with carmine-marked stools, formed only part of this excretion.

Calculations of the endogenous nitrogen content of infant stool agreed closely with those of previous workers.

The use of intravenously injected ¹⁵N showed that part of the stool nitrogen was derived from this source. It was excreted as urea, ammonia, and other compounds, and totalled about 2% of the administered urea.

The persistence of the ¹⁵N in the stool suggests that it had in part been incorporated into cellular material.

In studies of nitrogen absorption in man it is necessary to differentiate between faecal nitrogen derived directly from diet and that from endogenous sources. Many attempts have been made to measure the amount of endogenous nitrogen excreted in stool, and to calculate the unabsorbed dietary nitrogen difference (Murlin, Edwards, Hawley, and Clark, 1946). Such estimates of faecal endogenous nitrogen are suspect on the grounds that they have involved feeding diets very low, or entirely lacking, in nitrogen, and under such conditions the digestive system is unlikely to behave normally.

A different approach to the problem has been made by a few workers, who have used protein labelled with the non-radioactive heavy isotope of nitrogen with atomic mass 15 (White and Parson, 1950; Sharp, Lassen, Shankman, Gebhart, and Hazlet, 1956; Crane and Neuberger, 1960). The commonest protein so used has been that of *Saccharomyces cerevisiae* which is relatively easy to prepare and is well absorbed. This has been fed as whole yeast, or as partially purified protein, and the duration of the experiment kept sufficiently short to avoid rapidly synthesized body protein derived from the isotopic material being returned to the digestive tract through sloughed off cells or digestive enzymes. The first section of this study describes the use of ¹⁵N labelled yeast protein in an attempt to measure the level of endogenous faecal nitrogen excretion in two acutely malnourished and two recovered infants.

The second part of the study carried out on two marasmic and one recovered infant shows that the use of labelled protein is not suitable for accurate measurements of this nature, and that urea, a hitherto unsuspected source of endogenous faecal nitrogen in the non-ruminant, is rapidly formed from dietary protein, and is in part secreted into the digestive tract. Part of this urea nitrogen, mainly after further metabolism, is then excreted in the stool.

Experimental

SUBJECTS

The subjects were all male Arab infants between the ages of 3 and 12 months, and were either suffering from acute and severe energy protein malnutrition of the marasmic type or had fully recovered from the condition. A recently published system (McLaren and Read, 1972), weight/length/age, for estimation of the nutritional status of children was used to determine when the subjects were recovered. During the period of study, the infants were admitted to a metabolic ward, where they were under the care of a paediatrician. Consent was obtained from the parents of each child for its participation in the experiments, which had been passed by the Ethical Committee of this University.

MATERIALS

¹⁵N yeast protein of 51% purity, and containing 49.8% of its nitrogen as the heavy isotope, was prepared by a described method (Crane and Neu-

berger, 1960). The impurities in the preparation were non-nitrogenous. ^{15}N urea, with 96 atoms% of the isotope, was obtained from a commercial source. It was prepared by the hospital pharmacy as a 1.5% solution for intravenous use.

METHODS

During the course of each experiment the infant was nursed on a metabolic bed. Urine was collected at three hourly intervals, from a bag fixed to the perineum of the child, and after the addition of 1 ml of 5N HCl and 1 ml toluene, was frozen until required for analysis. Stool samples were collected as passed, the time of passing was recorded, and the specimen was frozen immediately. Special care was taken to see that no stool was contaminated with urine. Urine samples were collected for 72 hr after the beginning of the experiment and stool for 100 hr. The children were fed a commercial milk formula, providing approximately 3 g protein and 175 Calories per kg per 24 hr, in the acutely marasmic cases and 1.6 g protein and 120 Calories per kg per 24 hr for the recovered. For the yeast protein experiments, the labelled material was given by stomach tube in a dose of about 0.7 mg ^{15}N per kg immediately before the first meal of the first day of the experiment. For the urea experiment, the test material was injected in a dose of 1 mg ^{15}N per kg at a similar time. The meal given immediately after the test material was mixed with a little carmine, which acted as a stool marker.

ANALYTICAL PROCEDURE

All urine and stool specimens were assayed for total nitrogen by Kjeldahl analysis. In addition urine samples were analysed for urea by the diacetyl monoxime method (Kitamura and Iuchi, 1959) and for ammonia. In some experiments, stool samples after being homogenized with water were separated into water-soluble and insoluble components by pressure filtration through a fine pored filter. In one experiment, the filtrate was further filtered through a cellulose membrane, to remove water-

soluble material of high molecular weight. The water-soluble material was analysed for total nitrogen, urea, and ammonia. Water-soluble material retained by the cellulose membrane was analysed for protein.

All samples were analysed for ^{15}N content in a mass spectrometer (AEI MS10) after the prepared specimens were treated with sodium hypobromite to release nitrogen (Sprinson and Rittenberg, 1949). Samples for total ^{15}N assay were first digested as for Kjeldahl analysis, and the ammonia so formed was collected in dilute acid. Samples for urea ^{15}N analysis were used without further treatment after passing through a column of Dowex 50W resin to remove ammonia (Regoeczi, Irons, Koj, and McFarlane, 1965), and ammonia was distilled from samples after adjustment to pH 10 with potassium carbonate and collected in dilute acid.

Results

^{15}N YEAST PROTEIN EXPERIMENT

All stool samples passed after the administration of yeast protein contained measurable amounts of ^{15}N . A specimen passed only 2.5 hr after the test meal had 0.010 atoms% excess of ^{15}N , equivalent to 0.021 mg ^{15}N or 0.4% of the dose. The first carmine-labelled stool of this subject was passed 18 hours later. Similar amounts of ^{15}N were found in all stools passed throughout the experiment, except for those marked with carmine. These contained very much more isotope. Similar results were obtained from all subjects, although the widely differing intervals between the passing of stools make accurate comparison impossible. The mean excretion of ^{15}N in the carmine-marked stools was $6.33 \pm 1.3\%$ of the ingested material. No difference was apparent between marasmic and recovered subjects. Total nitrogen excreted in the marked stool amounted to 22.6%, 19.6%, 16.8%, and 17.4% of the food nitrogen given at the same time as the labelled yeast. The first two values are for marasmic children and the last two for recovered. Since it is unlikely

Subject	Marked Stool ^{15}N	Dietary N (mg/24 hr)	Exogenous N (mg/24 hr)		Total Faecal N (mg/24 hr)	Endogenous N (mg/24 hr)	Endogenous N (mg/kg/24 hr)
	Dietary ^{15}N		Marked Stool ^{15}N	$(\frac{\text{Marked Stool } ^{15}\text{N}}{\text{Dietary } ^{15}\text{N}} \times \text{Dietary N})$			
Marasmic 1 3.5 kg	0.1815	2730	495		616	121	34.6
Marasmic 2 4.5 kg	0.1583	4160	658		816	158	35.1
Recovered 1 4.4 kg	0.1320	2910	384		490	106	24.1
Recovered 2 8.5 kg	0.1230	3760	462		654	192	22.6

Table I Calculated 24-hr exogenous and endogenous faecal nitrogen excretion in four infants

Fig Typical excretion of ¹⁵N in stool after feeding ¹⁵N yeast protein

that milk protein nitrogen is more poorly absorbed than that from yeast protein, it is possible to calculate the minimum contribution of endogenous nitrogen to the total excretion (table I). The calculated endogenous faecal nitrogen expressed as mg/kg/day was found to be about 50% higher in the marasmic than in the control children.

The figure shows a typical ¹⁵N faecal excretion pattern for 100 hr after administration of the labelled

yeast protein. The very high level of isotope excreted with the stool marker is clearly illustrated.

¹⁵N UREA EXPERIMENT

Nitrogen derived from intravenously administered ¹⁵N urea appeared in all stools passed throughout the 72 hours of the experiments. Table II shows in detail the pattern of excretion of the isotope in one child. Table III shows the nitrogen and ¹⁵N excre-

Time after Injection (hr)	Urine Content (¹⁵ N mg)	Stool Content (¹⁵ N mg)	Water-insoluble Stool ¹⁵ N (mg)	Water-soluble Stool ¹⁵ N (mg)	Stool Urea ¹⁵ N (mg)	Stool Ammonia ¹⁵ N
1	1.655	0.021	0.013	0.008	0.005	0.001
3	3.818	0.040	0.020	0.020	0.008	0.005
6	2.456	0.068	0.030	0.038	0.015	0.005
9	1.245					
12	0.823					
15	0.386					
18	0.229					
21	0.241	0.029	0.013	0.016	0.006	0.004
24	0.177					
30	0.169	0.022	0.015	0.007	0.005	0.002
36	0.071	0.022	0.013	0.009	0.005	0.002
42	0.052	0.060	0.050	0.010	0.009	0.001
48	0.061	0.016	0.015	0.001	0.001	—
60	0.052	0.013	0.011	0.002	0.001	—
72	Trace	0.009	0.008	0.001	— ¹	—
Total	11.435	0.300	0.188	0.112	0.055	0.020

Table II Excretion of ¹⁵N in stool after ¹⁵N urea injection (15.596 mg) in one recovered subject

¹ — = Undetectable

Subject	Total Stool ^{15}N (mg)	Water-soluble ^{15}N (mg)	Water-insoluble ^{15}N (mg)	Total Stool N (mg)	Water-soluble N (mg)	Water-insoluble N (mg)
Marasmic						
0-24 hr	0.178	0.126	0.052	885	289	596
24-48 hr	0.105	0.025	0.080	668	227	441
48-72 hr	0.053	0.019	0.034	449	119	330
Total	0.336	0.170	0.166	2002	635	1367
Marasmic						
0-24 hr	0.070	0.032	0.038	378	154	224
24-48 hr	0.024	0.007	0.017	299	125	174
48-72 hr	0.020	0.014	0.006	322	133	189
Total	0.114	0.053	0.061	999	412	587
Recovered						
0-24 hr	0.180	0.089	0.091	947	398	549
24-48 hr	0.098	0.020	0.078	666	232	434
48-72 hr	0.022	0.003	0.019	154	90	64
Total	0.300	0.112	0.188	1767	720	1047

Table III Faecal nitrogen excretion following intravenous administration of ^{15}N urea

tion per 24 hours in each of the three subjects. The relationships between the total isotopic and normal nitrogen excretion, and the change from water-soluble to insoluble ^{15}N with time are apparent.

In one experiment, water-soluble material, which would not pass through a dialysis membrane, was separated. The amount was very small, but the biuret reaction showed the presence of protein and after Kjeldahl digestion of this material the ammonia formed had 0.012 atoms % excess of ^{15}N .

Discussion

The results of the ^{15}N yeast protein experiments show that nitrogen derived from this material and appearing in stool is excreted by at least three different routes.

Shortly after the material is ingested a low level of isotope enrichment appears in the stool, which seems to be mainly water-soluble material. This includes labelled urea and ammonia. At a later state, coinciding with the appearance of the carmine given with the test material, a much greater concentration of ^{15}N is present. This is almost certainly mainly due to unabsorbed ^{15}N from the yeast protein. In later, unmarked stools, the level of ^{15}N is similar to that of the earliest specimens, and there is a tendency for a higher proportion to be present in the water-insoluble fraction.

The most likely route of excretion of the rapidly appearing ^{15}N is via urea production from the test material. It is known that in man ^{15}N yeast protein is rapidly absorbed and metabolized, and that labelled urea is excreted in urine in less than 30 minutes (Crane and Neuberger, 1960). It is also known (Walser and Bodenlos, 1959) that consider-

able amounts of urea are secreted into the caecum, where bacterial ureases hydrolyse it to ammonia and carbon dioxide, which are then presumed to be quantitatively absorbed back into the body. Studies of faecal composition in adults (Wilson, Ing, Metcalfe, Gibson, and Wrong, 1968) have shown the presence of ammonia, but not measurable amounts of urea. The presence of urea in the stools of infants may be due to the shorter time that secreted urea would be in contact with bacterial ureases. Urea is poorly absorbed by the colon (Wolpert, Phillips, and Summerskill, 1971).

The ^{15}N excreted at the same time as the stool marker, although containing ^{15}N from urea, is mainly unabsorbed dietary nitrogen, and was used as a basis for calculation of maximum exogenous (and by difference, minimum endogenous) nitrogen excretion in stool. The figures in table I for the two recovered subjects agree closely with those obtained by Fomon, DeMaeyer, and Owen (1965) from infants of 4-6 months of age, who in a three-day balance study on a protein-deficient diet, excreted a mean of 23.7 mg/kg/day of nitrogen. The higher figure of 35 mg/kg/day for our marasmic subjects may have been due to their higher nitrogen intake. This agrees with the figure 32.5 mg/kg/day of Fomon *et al* for their infants on a higher protein intake. Differences in body composition between the two types of child might be responsible (Waterlow, 1969). The third type of excretion, in which much of the ^{15}N is contained as water-insoluble material, can best be explained by assuming that the isotope is incorporated into cells which are excreted in the faeces. Two main types of cell are present in stool: bacterial cells and sloughed off cells from intestinal villi. It seems unlikely in the limited period of the

experiment that the latter source could contribute very much to the ^{15}N excretion, but the rapidly growing intestinal flora which constitute over 30% of total stool solids are a possible main source. Many of these bacteria produce ammonia from urea (Walser and Bodenlos, 1959; Vince, Dawson, Park, and O'Grady, 1973) and it is not unreasonable to suppose that they may use some of this ammonia for amino acid synthesis. In ruminants, a major source of amino acids is from the bacteria in the rumen which after hydrolysing urea use the ammonia formed for amino acid synthesis.

To test the hypothesis that urea is a source of faecal nitrogen the second series of experiments was carried out. Except for the absence of the large peak of ^{15}N corresponding to excretion of unabsorbed material, a pattern of excretion of ^{15}N , similar to that from ^{15}N yeast, amounting to about 2% of the injected material was obtained (table II) demonstrating that urea was in part, at least, the source of the excretion of ^{15}N not due to unabsorbed food ^{15}N . The appearance of the ^{15}N from urea in the water-insoluble material (mainly cellular material) shows that urea and/or compounds derived from it are incorporated into this fraction. If the cells containing the ^{15}N are bacterial, as we suppose, then this is a previously unsuspected site of amino acid synthesis in the body. Although the colon is not capable of digesting the intestinal flora it can absorb amino acids and there is a high probability that a percentage of the amino acids synthesized by the bacteria will be released into the surrounding medium. Under conditions of starvation these, which would include essential as well as inessential amino acids, could provide the body with a small but valuable auxiliary supply of nutrients.

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