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Research Article

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Studies with ^{15}N-Labeled Ammonia and Urea in the Malnourished Child

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A B S T R A C T Investigations using ammonium citrate–^{15}N and urea-^{15}N showed that children in the acute stage of kwashiorkor and marasmus receiving a diet of adequate protein content retained a considerable percentage of the label from both compounds. Excretion of both total ^{15}N and urea-^{15}N was subnormal and elimination was virtually completed 36 hr after administration of the isotope. During recovery from kwashiorkor total ^{15}N excretion had approached normal a month after commencement of rehabilitation. Urea-^{15}N excretion was still slightly subnormal after 3 months. In marasmus urea-^{15}N formed a normal proportion of total ^{15}N excretion after 1 month, although total ^{15}N excretion then was still low. Ammonia nitrogen was retained to a greater extent than urea nitrogen in all cases. As it is known that a considerable amount of urea is degraded to ammonia in the gastrointestinal tract, it seems probable that urea nitrogen became available for use after this degradation. Examination of blood from one marasmic child after feeding ammonia-^{15}N and from another after intravenous injection of urea-^{15}N showed incorporation of the label into blood cells and plasma proteins. This did not occur in well nourished controls. It is concluded that ammonia and urea as sources of nonessential nitrogen may play an important part in protein metabolism in the malnourished child.

INTRODUCTION

For optimal growth the mammalian body requires a diet containing nitrogen in the form of essential amino acids, together with nitrogen derived from other sources. Although the amount and proportion of the essential amino acids required appear to be closely related to species and age, the source of nonessential nitrogen

seems to be relatively unimportant. Nonessential nitrogen is normally derived mainly from a mixture of nonessential amino acids.

In the young rat normal growth can continue (1) when ammonia is the only source of nonessential nitrogen supplementing a mixture of essential amino acids equivalent in quality and quantity to those known to be necessary for optimal growth. Several individual amino acids and urea are also capable of substituting for the usual dietary nonessential nitrogen in some animals (1).

Studies with ammonia-^{15}N in animals (2–4), healthy adults (2) and children (5), and adult patients with various diseases (2, 6) have shown that when dietary nitrogen is restricted a high proportion of the administered ammonia is retained. It is not clear whether the poor retention of ammonia nitrogen in the presence of an adequate diet is due to the preference of the body for other forms of nonessential nitrogen or whether it results from dilution with dietary nitrogen.

Normally over 90% of administered ammonia is excreted in less than 48 hr (2), suggesting that other forms of nitrogen are used preferentially. Conditions causing retention of ammonia nitrogen, as already described, lead also to urea nitrogen retention (1, 5, 7, 8) and subsequent protein synthesis (8). There is considerable evidence to show that in order for urea nitrogen to be utilized it must first be degraded to ammonia. The site of degradation is the digestive tract and is probably effected by ureases formed by the gastric and intestinal flora (9–12). In germ-free (13) and eviscerated (11) animals degradation of urea to ammonia ceases. Several studies provide evidence to show that various antibiotics or combinations of antibiotics inhibit (11) or stop (9, 14) this process. Low protein intake in the rat resulting in reduced urinary nitrogen excretion also reduces the activity of enzymes of the urea cycle (15). In the liver of malnourished children (16) the activity of argininosuccinase is reduced and that of amino acid–synthesizing enzymes increased.

In the severely malnourished child, in whom nitrogen intake is frequently below the minimum required to prevent body protein being catabolized, adaptation resulting in increased utilization of what are normally waste products would effect an economy of nitrogen and improve the prospects of recovery. The purpose of the present study was to determine to what extent the malnourished child might use ammonia and urea nitrogen under good dietary conditions, and the effect nutritional rehabilitation has on the extent of such utilization.

METHODS

Subjects

The subjects were Arab male children ranging between 3 and 14 months of age on admission. They presented with diarrhea, vomiting, various infections, and dehydration. All these symptoms were controlled in the hospital before the child was transferred to the Clinical Nutrition Unit for rehabilitation and study. Although no special study was made of hepatic, renal, or absorptive function, none of the subjects showed clinical evidence of disturbance. It is possible that mild impairment of renal function, known to occur (17), might have been partly responsible for the abnormal findings in the acutely malnourished children but this is not thought to be likely.

In the ammonia-^{15}N study there was one healthy control, one child with severe kwashiorkor, and six with varying degrees of marasmus. Three of the malnourished subjects were studied at intervals during recovery. For the patient with kwashiorkor, studies commenced on days 3, 38, 99, and 129, and for the two with marasmus, on days 3, 32, 65, and 98, and days 7 and 130, respectively. These children on recovery served as their own controls. In the urea-^{15}N study there were two healthy controls and four patients with marasmus.

The patient with kwashiorkor had on admission apathy, anorexia, extensive dermatosis, edema, hepatomegaly, and hair changes, typical clinical features of the condition. In addition there was evidence of advanced vitamin A deficiency (serum vitamin A 4 µg/100 ml and bilateral keratomalacia). The children with marasmus showed on admission marked wasting with loss of subcutaneous fat and skeletal muscle. In none was there edema or any of the signs of kwashiorkor. Subjects younger than 6 months received a cow's milk formula providing approximately 200 cal and 4 g of protein/kg per day. Older children received milk and Laubina (18) giving a daily intake of about 150 cal and 3 g of protein/kg. The patients stayed in the Unit for from 3 to 5 months and all made a good recovery, except for the permanent corneal scarring resulting in blindness in the subject with kwashiorkor. All attained their expected weight for height before discharge. The controls were admitted for 1 wk to the Unit from an orphanage specially for the study.

Isotope administration and sample collection

The subjects were nursed on a metabolic bed throughout the experimental period. The diet fed during the study was given for a minimum period of 48 hr before the study commenced in the acute state and considerably longer during recovery. A greater length of time would have been desirable to reach steady-state conditions but it was considered more important to obtain results in the acute stage before changes due to recovery were well established.

Ammonia-^{15}N study

Ammonia was given in the form of diammonium citrate prepared by a previously described method (2), from ammonium nitrate [1] containing approximately 30% of its nitrogen as the heavy isotope.

Two control subjects and six children with marasmus received diammonium citrate in aqueous solution by stomach tube immediately before the first feed of the day and as soon as possible after urine had been passed. The dose was approximately 5 mg/kg body weight. 50 mg carmine was incorporated into the following feed to act as a stool marker. 48 hr later 50 mg carmine was given with the feed to act as the second stool marker. Stools were collected, every precaution being taken to avoid loss, from the appearance of the first marker until the appearance of the second. Stools collected were pooled and frozen at −20°C until required for analysis. Urine was collected in a polythene bag adherent to the perineum. Quantitative removal of urine was made by aspiration and washing with distilled water at the following times after administration of the isotope: 3, 6, 9, 12, 15, 24, 30, 36, and 48 hr. Each urine sample was acidified with sulfuric acid, 1 ml of toluene was added as preservative, and the urine was stored immediately after collection at −20°C until required for analysis.

One marasmic child received diammonium citrate-^{15}N by stomach tube in a dose of 5 mg/kg body weight every day for five days. Venipuncture samples were collected at weekly intervals for 7 wk from the commencement of the experiment. The blood was collected into heparinized tubes and the plasma separated by centrifugation. The red cells were washed twice with normal saline. Both erythrocytes and plasma were then stored at −20°C until analyzed.

Urea-^{15}N study

The subjects were two control and three marasmic children. After the subject had passed urine he was given intravenously 4 mg/kg body weight of urea having 96% of its nitrogen as the heavy isotope [2] in the form of 1.2% solution in sterile saline. Urine was collected at 3-hr intervals for 48 hr after administration of the dose. Each sample was preserved with toluene and frozen at −20°C immediately after collection.

In one marasmic child 12 mg urea-^{15}N was given intravenously as a 1.2% solution in normal saline on each of 3 successive days. 1 wk after commencement of the experiment blood was taken by venipuncture, and treated as described previously.

Analysis of samples

Feces. Samples were homogenized with water and aliquots were analyzed for content of total nitrogen and of ^{15}N. Total fecal nitrogen was measured by Kjeldahl analysis, using mercuric sulfate as catalyst. For isotope measurement ammonia formed from Kjeldahl digestion was steam-distilled into 0.02 N sulfuric acid and the solution frozen until required.

Urine. Each sample of urine from both studies was analyzed for total nitrogen, ammonia nitrogen, and urea nitrogen. Analysis of the samples from the urea study was carried out within 48 hr of the last collection to minimize possible degradation of urea to ammonia. Urinary nitrogen was measured and specimens prepared for isotope analysis as described above for fecal nitrogen.

TABLE I
Nitrogen Balance over 48 hr of Subjects in Ammonia-¹⁵N Study

Subject	Day of study	Dietary nitrogen	Fecal nitrogen	Urinary nitrogen	Nitrogen retained	Nitrogen retained as per cent of intake	Per cent of urinary nitrogen as urea
		g	g	g	g	%	%
Control 1	—	16.21	1.02	7.44	7.75	47.8	80.2
Kwashiorkor	3	3.07	0.42	0.88	1.77	57.7	43.5
	38	7.91	1.47	6.86	−0.42	—	80.7
	99	10.25	0.85	7.75	1.65	16.1	79.1
	129	14.06	1.06	9.74	3.26	23.2	71.0
Marasmus	3	4.12	1.30	1.74	1.08	26.2	62.5
	32	5.92	0.79	2.20	2.93	49.5	68.0
	65	4.40	0.82	3.51	0.07	1.6	67.4
	98	6.03	1.17	2.66	2.20	36.5	66.1
Marasmus	7	3.16	0.41	0.93	1.82	57.6	47.5
	130	9.96	1.29	3.28	5.39	54.1	70.5
Marasmus	15	5.90	1.47	1.53	2.90	49.2	56.6
Marasmus	84	6.97	0.85	2.41	3.71	53.2	51.6
Marasmus	23	4.40	0.90	1.35	2.15	48.9	64.6

Urinary ammonia was collected by aspiration with nitrogen into acid solution, after adjustment of the sample to pH 10 with saturated sodium tetraborate. Total ammonia was determined by titration and aspirates for isotope measurement were frozen until required for analysis. Samples of urine for total urea analysis were adjusted to pH 7, incubated with 25 mg urease[3] at 37°C for 2 hr and the resulting ammonia then aspirated into acid solution as described for ammonia. The urea concentration was derived by difference. Urine samples for urea isotope measurements were used directly.

Nitrogen was released from the samples prepared for isotope assay and from 1 ml of each urine sample by reaction with alkaline sodium hypobromite (2). The resulting nitrogen was admitted to a commercial symmetrical single focusing mass spectrometer.[4] The mean value of 10 readings of the relative abundance of masses 28, 29, and 30 was used for calculation of the excess of ¹⁵N in the specimens.

Blood. Water-insoluble proteins were precipitated from the plasma by dialysis against running water. Blood cells and water-insoluble and water-soluble proteins of plasma were each subjected to Kjeldahl digestion and the ¹⁵N content determined in the mass spectrometer.

RESULTS

Table I shows the nitrogen balance of each subject in the ammonia-¹⁵N study for each 48 hr period of study. The percentage of urinary nitrogen excreted as urea is also shown. In all studies except one, there was positive nitrogen balance. The exception was probably caused by mild infection which became apparent 2 days after the

balance was completed, but absorption and retention of ¹⁵N were not affected. In the acutely malnourished state urea excretion was low, but returned to a normal figure during rehabilitation. Low urinary nitrogen excretion was, in all cases where it occurred, due to low urea output.

Table II shows the fate of ¹⁵N administered as ammonium citrate. In all subjects absorption of the ammonium citrate was high. In the acutely malnourished state the subjects with marasmus and kwashiorkor retained considerable amounts of the ¹⁵N. During recovery the

TABLE II
Fate of Absorbed ¹⁵N from Ammonium Citrate 48 hr after Administration

| Subject	Day of study	¹⁵N absorbed	¹⁵N lost as urea	¹⁵N lost as ammonia	Total urinary loss	¹⁵N retention
		%	%	%	%	%
Control 1	—	98.5	55.2	2.8	96.0	4.0
Kwashiorkor	3	100	9.5	3.9	22.3	77.7
	38	100	36.5	0.6	52.2	47.8
	99	94.0	46.5	0.7	61.0	39.0
	129	95.7	45.9	2.3	92.3	7.7
Marasmus	3	100	18.1	2.6	29.8	70.2
	32	95.3	27.5	5.7	77.6	22.4
	65	94.4	58.2	4.7	90.7	9.3
	98	96.3	47.0	3.1	82.1	17.9
Marasmus	7	100	19.0	2.2	35.0	65.0
	130	92.4	54.3	3.6	78.8	21.2
Marasmus	15	65.3	43.5	6.3	76.7	23.3
Marasmus	84	90.0	56.2	3.2	95.8	4.2
Marasmum	23	94.9	54.0	11.6	89.6	10.4
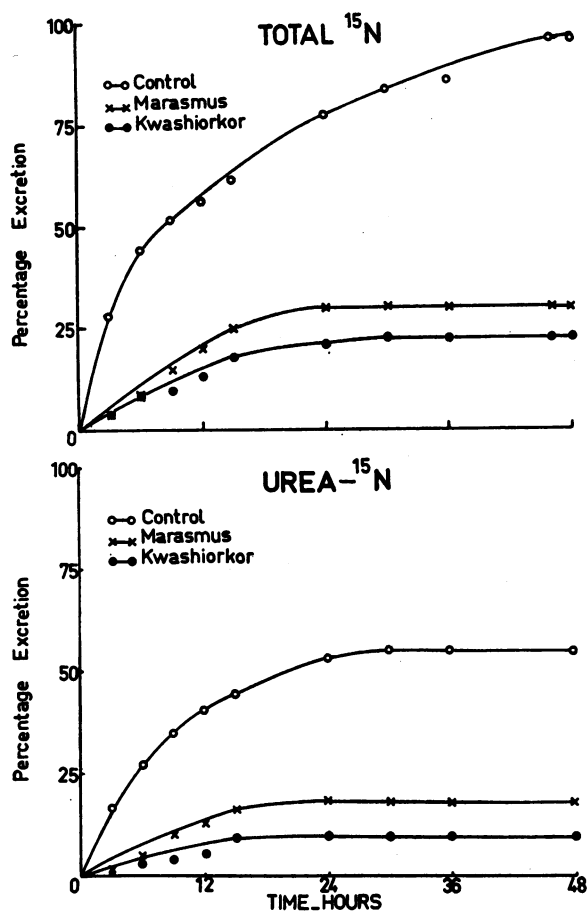
FIGURE 1 Excretion of total ^{15}N and urea-^{15}N in the urine of a healthy child, one with marasmus and one with kwashiorkor. Values expressed as percentage of ^{15}N administered as diammonium citrate.

retention decreased until it reached a similar level to that in the control. A feature of the subject with kwashiorkor in the acute stage was the relatively higher percentage loss of the label as ammonia than in other cases.

Fig. 1 shows the cumulative excretion of ^{15}N in urine over 48 hr as total ^{15}N and urea-^{15}N. Relatively small percentages of the absorbed ^{15}N dose were eliminated in the malnourished children giving as high a retention of the labeled nitrogen as 70% in marasmus and 77% in kwashiorkor.

Excretion of total ^{15}N in the control continued throughout the experimental period while in both marasmus and kwashiorkor isotope elimination ceased by the end of the 1st 24 hr. The low total output of the label in the malnourished children was accompanied by a reduced formation of urea-^{15}N. Elimination of urea-^{15}N ceased earlier in the patients than in the control children.

Figs. 2 and 3 show, respectively, the cumulative percentage excretion of total ^{15}N and urea-^{15}N in a normal subject and children with kwashiorkor and marasmus during rehabilitation. In marasmus return to normal of total ^{15}N excretion occurred more rapidly than in kwashiorkor.

^{15}N excretion as urea reached a normal level more slowly in marasmus than in kwashiorkor. This is in contrast to the pattern found for total ^{15}N elimination.

Labeled ammonium citrate fed over 5 days to one acutely marasmic subject resulted in appreciable amounts of the label appearing consistently in the blood cells and water-soluble and water-insoluble proteins of plasma as shown in Table III.

The urea-^{15}N study was carried out on two normal and four marasmic children. Table IV shows the total amount of ^{15}N excreted in the urine, and in the urinary urea and ammonia fractions as percentages of the administered isotope. The amount of ^{15}N excreted in forms other than urea in three acutely marasmic subjects suggests that there is considerable metabolism of urea nitrogen by these subjects. The very low excretion of ^{15}N as ammonia is in contrast to the results obtained for the subjects given ammonia, in whom several per cent was excreted by this route.

Fig. 4 shows the rate of excretion of ^{15}N in a control and in a typical acutely marasmic child. Of the total of 89% of the administered urea nitrogen excreted by the end of 48 hr by the control, almost 80% had been excreted at the end of 24 hr. The relative amounts excreted as urea at these times were 75 and 67%. In the marasmic subject the amounts of ^{15}N excreted at the same times were 70 and 56% for total nitrogen, and 40 and 30% as urea.

When a urea molecule with both nitrogen atoms of the heavy variety is hydrolyzed, two molecules of $^{15}NH_3$ are formed. If urea is resynthesized from these molecules in the presence of a preponderance of unlabeled ammonia there is only a small chance that two labeled molecules would be incorporated into one urea molecule. It has been shown that when nitrogen molecules are formed by reaction of urea with sodium hypobromite, both atoms of the nitrogen molecule are derived from a single urea molecule (19). It is thus possible, by measuring the ratio of nitrogen molecules of masses 29 and 30, to estimate the rate at which newly formed urea is being produced from ammonia derived from administered labeled urea (20). Fig. 5 shows the change with time of the ratio of nitrogen molecules with weights 30 and 29 formed from urinary urea by reaction with sodium hypobromite. In the normal child the ratio $^{15}N^{15}N : ^{14}N^{15}N$ decreased in an exponential fashion showing that a constant proportion of body urea was being degraded and then reformed into urea. The ratio decreased by 50% in 9 hr. In the marasmic child the change in the ratio was quite different. It did not decrease exponentially and the lowering of the ratio proceeded at a greater rate.
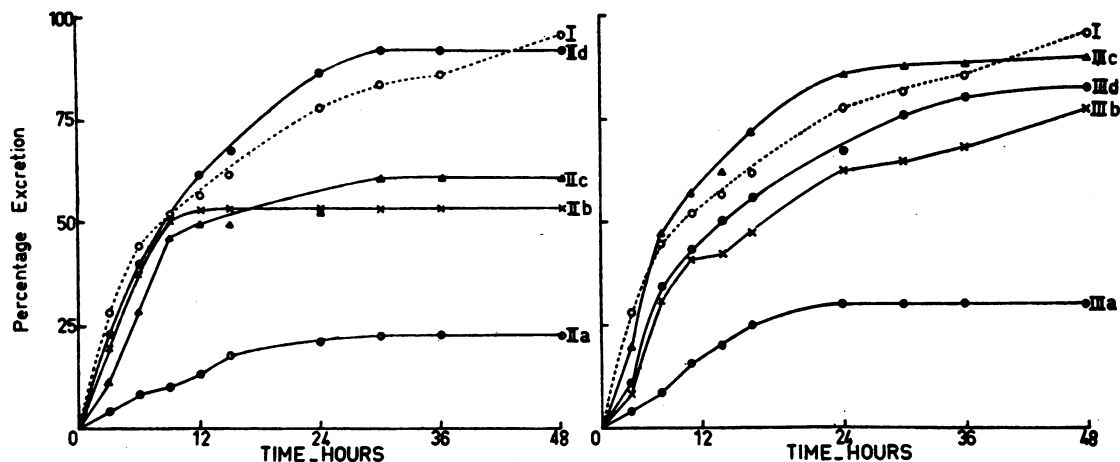
FIGURE 2 Changes in urinary excretion of total ¹⁵N after administration of diammonium citrate-¹⁵N during rehabilitation of a child with kwashiorkor (*II*) and one with marasmus (*III*). Values for one control (*I*) are given for comparison. The letters *a–d* for the malnourished subjects indicate the day of the study (*a* = day 3; *b* = day 38 or 32; etc., as indicated in Table II).

Examination of the blood components from a marasmic child given urea-¹⁵N showed the presence of 0.011 atoms % excess in the cells and in the water-insoluble proteins from plasma. The water-soluble plasma proteins contained 0.012 atoms % excess of ¹⁵N. In a control subject no detectable excess of ¹⁵N was found.

DISCUSSION

The results of the investigation show clearly that the acutely malnourished child, with either kwashiorkor or marasmus, retains a high percentage of ¹⁵N when the isotope is administered as ammonium citrate. The normal child excretes most of it within 48 hr. Since there is every reason to believe that ¹⁵N and the commoner

form of nitrogen are treated similarly by the body, it may be assumed that ammonia nitrogen derived from deamination of amino acids is also retained in acute malnutrition. The usual fate of such ammonia nitrogen is to be excreted in the form of urea. In malnourished children, however, urea excretion is low, and considerable amounts of nitrogen derived from ammonia are found in blood proteins where the label persists for a considerable period of time. Alterations in activity of liver enzymes recently reported to occur in malnourished children would help to explain such changes (16). In the healthy child almost all of the administered isotope was excreted within 48 hr and none could be detected in the blood. It has been suggested that incorporation of ¹⁵N into se-

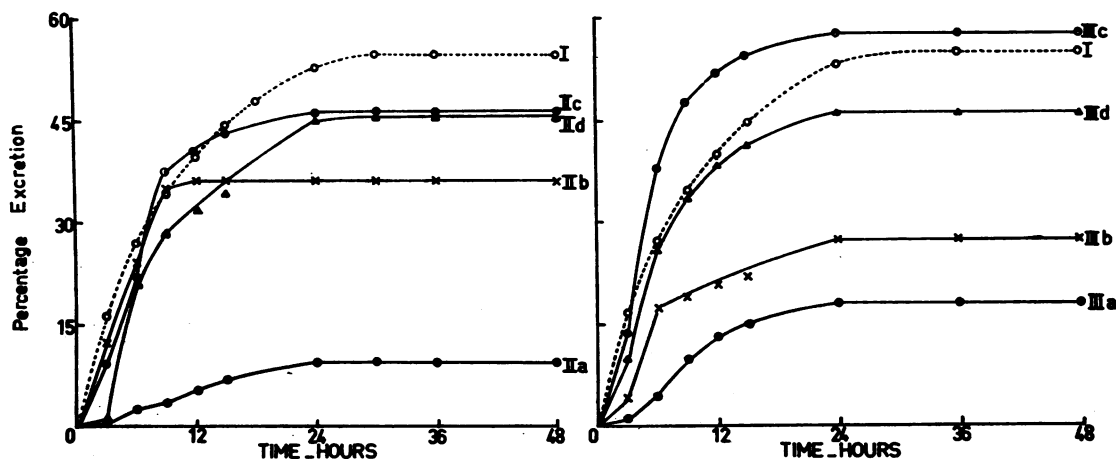FIGURE 3 Changes in urinary excretion of urea-¹⁵N after administration of diammonium citrate-¹⁵N in the same subjects as Fig. 2.

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TABLE III

Concentration of ^{15}N in Some Blood Components of One
Marasmic Subject of the Ammonia-^{15}N Study

Weeks after commencement of study	Atoms % excess of ^{15}N in			
	Blood cells	Plasma	Water-insoluble plasma proteins	Water-soluble plasma proteins
1	0.019	0.024		
3	0.012		0.015	0.016
4	0.011		0.012	0.010
5	0.008		0.010	0.012
6	0.010		0.009	0.002
7	0.003		0.009	0.005

TABLE IV

Urinary ^{15}N Excretion at the End of 48 hr
in the Urea-^{15}N Study

Subject	Day of study	% total ^{15}N	% ^{15}N as urea	% ^{15}N as ammonia	% ^{15}N in other compounds	% ^{15}N retained
Control 1	—	89.1	75.4	1.2	12.5	10.9
Control 2	—	98.8	81.0	0.7	17.1	1.2
Marasmus	15	75.4	42.3	1.0	33.0	24.6
Marasmus	7	84.3	63.9	0.6	19.8	15.7
Marasmus	10	69.1	40.5	0.6	28.6	30.9

rum albumin after oral administration of ammonium chloride-^{15}N to uremic subjects could be an exchange phenomenon (21). If this were taking place to any extent incorporation of the label should have been observed in the normal subject as well as in the malnourished.

If our results are typical, the child under conditions of extreme malnutrition is able to effect a reduction of nonessential nitrogen excretion amounting to several grams per day. That this happens even when a diet containing protein which supplies sufficient essential nitrogen to restore growth is fed, suggests that the total requirement for nonessential nitrogen is not being met by the diet. The concept of other workers (5) that nonessential nitrogen could be a limiting factor for human growth is in agreement with our findings.

The similarity of behavior of all the malnourished subjects with regard to nitrogen retention and excretion shows that the marasmic child has a requirement for nonessential nitrogen almost equal to that found in kwashiorkor. It is evident that marasmus is not just calorie deficiency, as has been suggested (22), and the present study provides further justification for retaining the term protein-calorie malnutrition to include kwashiorkor, marasmus, and intermediate forms (23).

The results with urea-^{15}N show that this form of nonessential nitrogen is also available for protein synthesis, although probably to a smaller degree than is ammonia. As far as we know, in order for this to take place urea must first be degraded to ammonia, and as other workers have shown considerable amounts of urea are so converted in the gut. Our results with ammonia-^{15}N, mentioned above, suggest that ammonia so formed is not all reconverted into urea in the malnourished child. That much is used for other purposes is shown by the fact that when ^{15}N is administered as urea a greater amount of label is found in the urinary nonurea nitrogen in the malnourished children than in the normal. It is of interest to note that the percentage of ^{15}N excreted and its distribution among the nitrogenous components are very similar in our normal infants and in the nor-

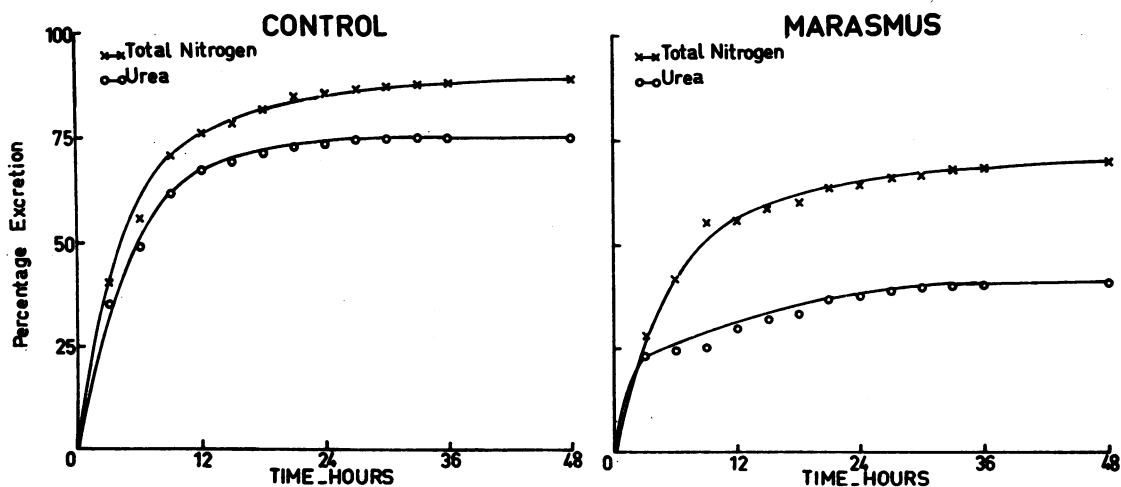FIGURE 4 Excretion of total ^{15}N and urea-^{15}N in the urine of a control child and one with marasmus. Values expressed as percentage of ^{15}N administered as urea.
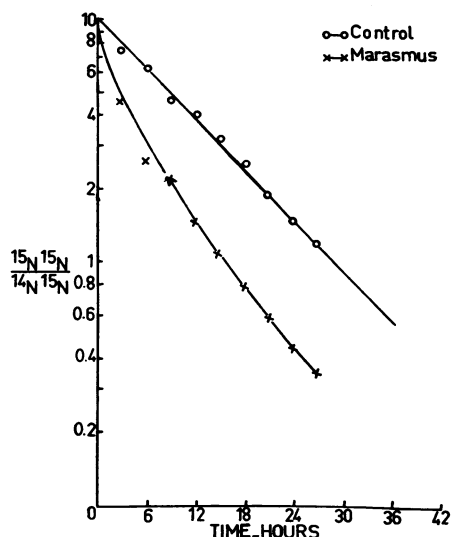
FIGURE 5 Change with time of the ratio $(^{15}N\,^{15}N)/(^{14}N\,^{15}N)$ in urinary urea after administration of urea-^{15}N in the same subjects as in Fig. 4.

mal adult (20). The use of urea with a very high content of ^{15}N and comparison of the amounts of nitrogen formed from urinary urea with molecular weights of 30 and 29 (19, 20) showed that a significant fraction of excreted urea-^{15}N had been newly synthesized in the control and the marasmic child. Comparison of the rate of change of the ratio in the malnourished subject, with that in the control shows that resynthesis of urea is proceeding at different rates in the two subjects. The data do not, however, suggest an explanation of the difference.

Since there is evidence that some broad-spectrum antibiotics may destroy bacterial flora responsible for producing ureases (9, 11, 14, 20), use of such compounds may indirectly have an adverse effect on the nitrogen-economizing systems which appear to operate in the malnourished child, and therefore increase the time necessary for rehabilitation.

ACKNOWLEDGMENTS

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