N-Acetyl-L-Tyrosine as a Tyrosine Source during Total Parenteral Nutrition in Adult Rats

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ABSTRACT. The tyrosine content of parenteral solutions is limited by poor tyrosine solubility. N-acetyl-L-tyrosine has excellent solubility and is a potential source of intravenous tyrosine. Infusion of N-acetyl-U-¹⁴C-L-tyrosine as part of a total parenteral nutrition regimen in the rat at a level of 0.5 mmol/kg/day resulted in rapid labeling of tissue tyrosine pools, production of ¹⁴CO₂, incorporation of ¹⁴C-labeled tyrosine into protein, and modest urinary losses (8.3%). Plasma tyrosine levels, however, remained at fasting values (73.8 ± 5.40 μM). Infusion of N-acetyl-L-tyrosine at 2 mmol/kg/day increased plasma tyrosine above fasting levels (141 ± 16.1 μM), resulted in a rapid labeling of tissue tyrosine pools, production of ¹⁴CO₂, and incorporation of ¹⁴C-labeled tyrosine into protein. However, urinary losses were higher (16.8%). Rapid utilization of N-acetyl-L-tyrosine was noted at both infusion levels. Plasma- and tissue-free tyrosine pools were rapidly labeled, as was tissue protein. Radioactivity incorporated in tissue protein was shown to be tyrosine after acid hydrolysis. (*Pediatr Res* 19: 514-518, 1985)

Abbreviations

N-Ac-Tyr, N-acetyl-L-tyrosine
Ala-Tyr, L-alanyl-L-tyrosine

Cystine and tyrosine have been suggested as essential amino acids for some premature infants (1-3). Both amino acids have limited solubility, and cannot be added to total parenteral nutrition solutions in quantities sufficient to meet the infant requirements. The absence of these amino acids results in depressed cystine and tyrosine levels in many infants infused with such solutions (4-6).

Soluble peptides of cystine and tyrosine are potential sources of these amino acids for inclusion in parenteral solutions (7). We have previously demonstrated (8, 9) good utilization of parenterally infused Ala-Tyr as a tyrosine source in adult rats when administered at either 0.5 or 2.0 mmol/kg body weight per day. The infused peptide was utilized as a tyrosine source for protein synthesis, as evidenced by incorporation of tyrosine into tissue protein and by an increase in plasma and tissue tyrosine concentrations, and for energy production as evidenced by rapid oxidation to carbon dioxide. However, tyrosine peptides are relatively expensive to synthesize in the amounts needed for the

parenteral infusion of human infants. Accordingly, we tested whether N-Ac-Tyr, a less expensive soluble tyrosine derivative, was utilized as a tyrosine source during parenteral nutrition in adult rats.

MATERIALS AND METHODS

N-acetyl-L-U-¹⁴C-tyrosine was prepared from U-¹⁴C-L-tyrosine (New England Nuclear, Boston, MA) and acetic anhydride by the method of Sheehan and Yang (10) and purified by recrystallization from ethyl acetate-benzene mixtures. The composition of the compound was checked by amino acid analysis before and after acid hydrolysis (6 N HCl, 110° C, 24 h).

Healthy male Sprague-Dawley rats weighing 300 to 400 g were used for all experiments. Utilization of N-Ac-Tyr was first tested in five animals after intraperitoneal injection of 0.5 mmol of N-Ac-Tyr per kilogram body weight [15 μCi N-acetyl-L-(U-¹⁴C)tyrosine] dissolved in 1 ml of isotonic saline. After injection, the rats were immediately placed in a glass metabolism chamber and the labeled carbon dioxide collected as described by Palese and Tephly (11) at 0.5, 1, 2, 4, 8, 12, and 24 h.

Since studies of N-Ac-Tyr injected intraperitoneally showed reasonable utilization, the compound was also studied when infused as part of a parenteral nutrition regimen. Rats were cannulated using the method of Popovic and Popovic (12) under ether anesthesia and allowed to recover from the operative procedure for a 2-day period. This cannulation procedure allows simultaneous sampling of arterial blood while the nutrient solution is infused into the venous circulation.

After recovery, the animals were placed in restraining metabolism cages and alimanted parenterally using the solution described by Steiger *et al.* (13). The base solution was prepared by adding sterile 50% dextrose solution to an 8.5% solution of crystalline amino acids (FreAmine, McGaw Laboratories, Glendale, CA) 2:1. This solution (33% glucose-2.8% amino acids) does not contain tyrosine. The solution was infused into the venous cannula at a rate of 50 to 55 ml/kg/day by pump (Extracorporeal Medical Specialties, Bridgeport, PA). Total daily energy intake was between 73 to 80 kcal, sufficient to provide maintenance requirements of adult rats (13, 14).

Three groups of five animals each were studied. The first group (group I) was infused with the parenteral solution without added N-Ac-Tyr. The second and third groups were infused with the same parenteral solution to which N-Ac-Tyr was added at levels providing either 0.5 (group II) or 2.0 (group III) mmol/kg body weight per 24 h infusion. Sufficient N-acetyl-U-¹⁴C-L-tyrosine was added so that 15 μCi were infused per 24 h in each case. The solutions were sterilized by passage through a 0.22-μ membrane filter (Millipore Corporation, Bedford, MA) before infusion.

Sequential 4-h urine samples were collected in iced graduate cylinders during the infusion, and deproteinized immediately with sulfosalicylic acid (15). Blood samples (0.5 ml) were drawn

Received November 5, 1984; accepted January 24, 1985.

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This research was supported in part, by a grant-in-aid from the National Foundation-March of Dimes.

from the arterial cannula at 0 and 24 h, heparinized, and centrifuged to separate plasma and erythrocytes. Plasma and erythrocyte samples were prepared and analyzed for amino acid content as described previously (8, 9). Simultaneous radioactivity and amino acid analyses were carried out as described by Stegink (16).

At the end of the parenteral infusion, the animals were lightly anesthetized with ether, and tissue samples (liver, kidney, muscle, intestine, brain, stomach, heart, lung, spleen) were quickly excised, blotted, and weighed. The remaining carcass was skinned. One-gram portions of each tissue were homogenized in 10 ml of 5% sulfosalicylic acid for 3 min in a Virtis microhomogenizer. The precipitated protein was removed by centrifugation at $20,000 \times g$ for 15 min at 4°C in a refrigerated centrifuge (International B-20), and aliquots of the supernatant solution were assayed for radioactivity and amino acid distribution as described by Stegink (16). The entire skin and remaining carcass were similarly treated.

The total radioactivity present in plasma, urine, tissue, and carcass homogenates was determined by adding aliquots of the homogenate to 10 ml of scintillation fluid (17). The values were quench corrected. Background noise was eliminated by counting an appropriate portion of control urine, plasma, or tissue homogenates. The quantity of radioactivity present in protein fractions of the samples was determined by dissolving a specific weight of precipitated protein in hyamine hydroxide (Packard Instrument Co., Downers Grove, IL) followed by digestion for 48 h at 37°C before counting.

The distribution of radioactivity between the various amino acids in the protein fraction of plasma and tissues was determined after acid hydrolysis. The precipitated protein (from the sulfosalicylic acid homogenate) was collected by centrifugation, suspended in 5 ml of 3.5% sulfosalicylic acid, and the protein was again collected by centrifugation. The supernatant solution was discarded and the procedure repeated three times to remove any traces of free tyrosine or N-Ac-Tyr. The precipitated protein was then dissolved in 6 N HCl in a hydrolysis tube. The tube was evacuated using a vacuum pump and then flushed with dry, oxygen-free nitrogen gas. After repeating the process three times, the tube was sealed and the protein hydrolyzed for 22 h at 115°C . The hydrolysate was lyophilized to dryness, the residue dissolved in 5% sulfosalicylic acid, and this solution then subjected to the simultaneous radioactivity-amino acid analysis procedure (16).

Statistical analysis of the data was carried out using the paired *t* test and the Student's *t* test (18).

RESULTS

Utilization of N-Ac-Tyr was first tested by intraperitoneal injection of 0.5 mmol/kg body weight N-Ac-Tyr into five adult rats. The results were compared with those obtained in previously studied animals (8) injected with an equimolar quantity of Ala-Tyr. The rate of ^{14}C -carbon dioxide release from animals injected with these two compounds is shown in Figure 1. Injection of either compound resulted in rapid labeling of expired carbon dioxide. Radioactive carbon dioxide release peaked within 30 min after injection and declined rapidly. However, the rate of ^{14}C -carbon dioxide release was more rapid with Ala-Tyr than with N-Ac-Tyr.

Table 1 shows the radioactivity distribution 24 h after intraperitoneal injection of N-Ac-Tyr. In general results were similar to those obtained earlier with Ala-Tyr; 41.2% of the label present in N-Ac-Tyr was expired as carbon dioxide and 8.31% was excreted in urine. The carcass accounted for most of the remaining label. Urinary losses were higher in animals given N-Ac-Tyr than those noted previously in animals injected with Ala-Tyr (4.6%). When the distribution of radioactivity in the urine of animals injected with N-Ac-Tyr was examined, 90% of the radioactivity in the urine was present as N-Ac-Tyr, while 10%

Fig. 1. Release of $^{14}\text{CO}_2$ from rats ($n = 5$ per group) injected intraperitoneally with $15 \mu\text{Ci}$ (0.5 mmol/kg body weight) of either Ala-Tyr (●—●) or Ac-Tyr (○—○). Values for animals injected with alanyl-tyrosine were published previously (8).

Table 1. Radioactivity distribution 24 h after intraperitoneal injection of 0.5 mmol/kg body wt of N-Ac-Tyr

Component	% of infused label in tissue or fluid
Carbon dioxide	$41.2 \pm 3.21^*$
Urine	8.31 ± 1.13
Carcass	45.2 ± 6.21
Liver	2.05 ± 0.52
Kidney	0.29 ± 0.11
Muscle	20.9 ± 2.31
Intestine	1.82 ± 0.59
Plasma	4.98 ± 2.61
Brain	0.07 ± 0.04
Stomach	0.39 ± 0.06
Heart	0.11 ± 0.02
Lung	0.17 ± 0.03
Spleen	0.05 ± 0.01
Feces	0.71 ± 0.26
Skin	0.91 ± 0.18
Remaining carcass	12.8 ± 5.21

* Values listed as the mean $\pm$ SD.

was present as free tyrosine. In animals injected with Ala-Tyr, 92% of the radioactivity in urine was present as tyrosine and only 8% as Ala-Tyr (8). Urinary free tyrosine excretion was significantly higher ($p < 0.05$) in rats injected with N-Ac-Tyr ($0.29 \pm 0.08 \mu\text{mol}/24 \text{ h}$) than in control rats injected with isotonic saline ($0.17 \pm 0.09 \mu\text{mol}/24 \text{ h}$).

Concentrations of free amino acids in plasma 24 h after injection did not differ significantly between control animals injected with isotonic saline and test animals injected intraperitoneally with N-Ac-Tyr.

Since the data obtained after intraperitoneal injection were promising, N-Ac-Tyr utilization was investigated when infused at 0.5 and 2.0 mmol/kg body weight as part of a total parenteral nutrition regimen. The results obtained were compared with values obtained earlier (8, 9) in animals treated with a similar regimen to which Ala-Tyr had been added at 0.5 and 2 mmol/kg body weight as the tyrosine source.

Studies were first carried out in animals infused with N-Ac-Tyr at 0.5 mmol/kg body weight (group II). Plasma amino acid levels, both before parenteral alimentation, and after 24-h infusion are shown in Table 2. In general, plasma levels of those amino acids present in the parenteral solution increased from fasting levels noted in preinfusion samples to levels considered postprandial in samples taken after 24 h infusion. Plasma levels

Table 2. Mean ($\pm$ SD) plasma amino acid levels (μ M) in rats undergoing total parenteral alimentation [total parenteral nutrition (TPN)] with and without added N-Ac-Tyr at 0.5 and 2.0 mmol/kg body wt*

Amino acid	TPN alone		TPN + 0.5 mmol/ kg N-Ac-Tyr		TPN + 2 mmol/ kg N-Ac-Tyr	
	0 time	24 h	0 time	24 h	0 time	24 h
Taurine	128 $\pm$ 42.7	122 $\pm$ 26.0	116 $\pm$ 27.7	282 $\pm$ 89.1	116 $\pm$ 27.7	185 $\pm$ 19.1
Aspartate	4.10 $\pm$ 1.80	4.20 $\pm$ 2.20	4.40 $\pm$ 1.40	7.60 $\pm$ 3.60	4.40 $\pm$ 1.40	5.42 $\pm$ 5.15
Threonine†	166 $\pm$ 27.8	228 $\pm$ 73.3	136 $\pm$ 25.5	190 $\pm$ 46.2	136 $\pm$ 25.5	239 $\pm$ 38.0
Serine	198 $\pm$ 43.7	273 $\pm$ 67.1	133 $\pm$ 22.2	197 $\pm$ 43.2	133 $\pm$ 22.2	362 $\pm$ 55.2
Glutamine-asparagine	442 $\pm$ 45.0	401 $\pm$ 21.6	472 $\pm$ 62.1	407 $\pm$ 61.0	458 $\pm$ 37.5	406 $\pm$ 42.1
Glutamate	45.7 $\pm$ 17.8	62.3 $\pm$ 23.9	64.4 $\pm$ 11.8	66.0 $\pm$ 39.4	64.4 $\pm$ 11.8	81.6 $\pm$ 14.0
Proline†	127 $\pm$ 9.50	196 $\pm$ 35.6	135 $\pm$ 14.4	198 $\pm$ 56.0	135 $\pm$ 14.4	237 $\pm$ 25.4
Citrulline	22.7 $\pm$ 13.1	21.1 $\pm$ 11.6	30.9 $\pm$ 15.7	32.6 $\pm$ 9.30	30.9 $\pm$ 15.7	44.3 $\pm$ 12.8
Glycine†	301 $\pm$ 171	525 $\pm$ 154	217 $\pm$ 49.3	397 $\pm$ 114	217 $\pm$ 49.3	499 $\pm$ 25.2
Alanine†	238 $\pm$ 48.1	364 $\pm$ 23.1	291 $\pm$ 14.7	441 $\pm$ 137	291 $\pm$ 14.7	536 $\pm$ 160
Valine†	168 $\pm$ 28.2	226 $\pm$ 68.7	107 $\pm$ 15.9	154 $\pm$ 26.4	107 $\pm$ 15.9	174 $\pm$ 23.0
1/2-Cystine	81.0 $\pm$ 24.5	71.3 $\pm$ 12.1	88.5 $\pm$ 3.60	87.3 $\pm$ 5.00	88.5 $\pm$ 3.60	86.2 $\pm$ 11.2
Methionine†	62.4 $\pm$ 12.2	130 $\pm$ 44.1	40.1 $\pm$ 6.40	77.1 $\pm$ 28.0	40.1 $\pm$ 6.40	98.1 $\pm$ 8.50
Isoleucine†	90.1 $\pm$ 24.9	123 $\pm$ 55.8	43.7 $\pm$ 12.2	61.7 $\pm$ 5.30	43.7 $\pm$ 12.2	72.8 $\pm$ 14.1
Leucine†	156 $\pm$ 37.8	199 $\pm$ 68.8	93.1 $\pm$ 25.9	112 $\pm$ 24.7	93.1 $\pm$ 25.9	126 $\pm$ 24.2
Tyrosine	76.9 $\pm$ 13.2	70.1 $\pm$ 18.4	59.1 $\pm$ 9.50	73.8 $\pm$ 5.40	59.1 $\pm$ 9.50	141 $\pm$ 16.1
Phenylalanine†	83.0 $\pm$ 13.2	126 $\pm$ 28.8	54.3 $\pm$ 9.60	98.5 $\pm$ 16.5	54.3 $\pm$ 9.60	105 $\pm$ 10.7
Ornithine	41.8 $\pm$ 6.00	49.5 $\pm$ 21.0	25.6 $\pm$ 9.50	36.8 $\pm$ 18.2	25.6 $\pm$ 9.50	53.9 $\pm$ 43.5
Lysine†	482 $\pm$ 157	678 $\pm$ 167	233 $\pm$ 52.8	410 $\pm$ 62.4	233 $\pm$ 52.8	549 $\pm$ 112
Histidine†	81.1 $\pm$ 36.4	111 $\pm$ 49.5	42.0 $\pm$ 9.90	80.7 $\pm$ 24.2	42.0 $\pm$ 9.90	91.1 $\pm$ 12.0
Arginine†	163 $\pm$ 10.0	217 $\pm$ 49.2	110 $\pm$ 16.6	180 $\pm$ 67.3	110 $\pm$ 16.6	169 $\pm$ 38.5

* Data shown as mean $\pm$ SD for the five animals studied in each group.

† Designates amino acids present in free form in the TPN solution.

Table 3. Radioactivity distribution after 24 h of total parenteral nutrition with 0.5 or 2.0 mmol/kg body wt of N-Ac-Tyr

Component	% of infused label in tissue or fluid	
	0.5 mmol/kg body wt	2.0 mmol/kg body wt
Carbon dioxide	36.3 $\pm$ 1.70*	25.6 $\pm$ 6.10
Urine	10.1 $\pm$ 2.46	16.8 $\pm$ 0.16
Carcass		
Liver	8.20 $\pm$ 1.67	5.97 $\pm$ 1.61
Kidney	2.78 $\pm$ 0.14	1.37 $\pm$ 0.24
Muscle	14.5 $\pm$ 1.40	8.92 $\pm$ 1.49
Intestine	3.55 $\pm$ 0.92	1.20 $\pm$ 0.48
Plasma	4.32 $\pm$ 0.39	5.97 $\pm$ 1.78
Brain	0.24 $\pm$ 0.05	0.15 $\pm$ 0.06
Stomach	1.01 $\pm$ 0.44	0.72 $\pm$ 0.05
Heart	0.21 $\pm$ 0.07	0.15 $\pm$ 0.03
Lung	0.55 $\pm$ 0.17	0.38 $\pm$ 0.04
Spleen	0.33 $\pm$ 0.03	0.25 $\pm$ 0.04
Feces	0.08 $\pm$ 0.02	0.08 $\pm$ 0.03
Skin	1.01 $\pm$ 0.36	1.55 $\pm$ 0.18
Remaining carcass	13.9 $\pm$ 6.17	17.4 $\pm$ 6.21

* Values listed as the mean $\pm$ SD.

of those amino acids not present in the parenteral solution remained at fasting levels or decreased. No differences in the response of these plasma amino acids were noted between solutions ($\pm$ N-Ac-Tyr). The addition of N-Ac-Tyr to the parenteral solution at 0.5 mmol/kg body weight/day did not significantly increase plasma tyrosine levels over baseline values. We had previously noted that Ala-Tyr infused at 0.5 mmol/kg body weight also failed to increase plasma tyrosine concentrations (9).

The infusion of radioactively labeled compounds made it possible to evaluate utilization of N-Ac-Tyr for both energy production (CO_2) and protein synthesis. The data in Table 3 demonstrate utilization of parenterally administered N-Ac-Tyr. Animals infused with 0.5 mmol/kg body weight N-Ac-Tyr ox-

dized 36% of the infused label, losing 10% in the urine. Of the radioactivity lost in urine, 74% was present as N-Ac-Tyr and 23% was present as tyrosine. Urinary losses of infused label were greater in these animals infused with N-Ac-Tyr than values noted when 0.5 mmol/kg body weight Ala-Tyr was used as the tyrosine source. In those previously studied animals (9), 42% of the infused label was oxidized and 7.7% was lost in the urine (74% was tyrosine and 26% Ala-Tyr).

These data clearly demonstrated utilization of N-Ac-Tyr as a tyrosine source for both tissue-free tyrosine pools and protein synthesis. After 24 h infusion of N-Ac-Tyr at 0.5 mmol/kg body weight, 53% of the infused label was present in tissue as either free or protein-bound tyrosine; muscle, liver, plasma, intestine, and kidney accumulated substantial amounts of radioactivity. The results are similar to those noted earlier in animals infused with Ala-Tyr at 0.5 mmol/kg body weight (9). Determination of the radioactivity distribution between the free amino acid fraction, the protein bound fraction, and the free N-Ac-Tyr content of the various tissue fractions showed 10 to 20% of the radioactivity present as free tyrosine, with the remainder in protein-bound form after 24 h infusion. Only trace quantities of radioactivity were detected at the free N-Ac-Tyr position. Isolation of protein from liver, plasma, and muscle extracts, followed by acid hydrolysis, and analysis for distribution of radioactivity among the various amino acids demonstrated that 94 to 99% of the radioactivity present in the protein was tyrosine. These data demonstrate incorporation of labeled intact tyrosine into protein. Labeling did not result from conversion of tyrosine into nonessential amino acids with subsequent incorporation of nonessential amino acids into protein.

Although good utilization of N-Ac-Tyr was noted when infused at 0.5 mmol/kg body weight as part of parenteral feeding regimen, the quantity of N-Ac-Tyr infused was not sufficient to increase plasma tyrosine concentrations above fasting levels (overall mean $67.8 \pm 7.42 \mu\text{M}$). Thus, group III animals were infused with a parenteral regimen providing N-Ac-Tyr at 2.0 mmol/kg body weight. Plasma amino acid concentrations in these animals are shown in Table 2. Plasma amino acid concentrations of those amino acids present in the parenteral mixture

increased during infusion, as noted in control rats infused with the parenteral nutrition solution alone without a tyrosine source, while levels of amino acids not present in the solution remained constant or decreased. Infusion of N-Ac-Tyr at 2.0 mmol/kg significantly ($p < 0.05$) increased plasma tyrosine concentrations over both baseline values and levels noted in control animals infused with the parenteral nutrition solution alone without an added tyrosine source. Plasma tyrosine concentrations increased from a mean baseline value of 71 ± 20 to $141 \pm 16 \mu\text{M}$ after 24 h infusion with 2 mmol/kg body weight N-Ac-Tyr. The latter value is similar to that noted in animals infused with 2 mmol/kg body weight Ala-Tyr ($114 \pm 21 \mu\text{M}$) (9).

The distribution of radioactivity in these animals is shown in Table 3. At this level of infusion 25.6% of infused N-Ac-Tyr was oxidized to carbon dioxide and 16.8% was excreted in the urine (82% N-Ac-Tyr; 18% tyrosine). The rate of tyrosine oxidation to carbon dioxide was lower and the rate of urinary loss greater than values noted in previously studied animals infused with Ala-Tyr at 2.0 mmol/kg body weight (9). Animals infused with 2 mmol/kg body weight Ala-Tyr oxidized 54% of infused label and excreted only 5.4% in the urine (45% Ala-Tyr, 55% tyrosine).

Radioactivity from N-Ac-Tyr was found in all tissues studied. Determination of the radioactivity distribution between the free amino acid fraction, the protein bound fraction, and free N-Ac-Tyr content of the various tissue fractions showed that 10 to 20% of the radioactivity was present as free tyrosine, with the remainder in protein-bound form after 24 h infusion. Only trace quantities of radioactivity were detected at the free N-Ac-Tyr position in most tissues. Plasma was an exception, and 15% of the total radioactivity was found in the free N-Ac-Tyr position. Isolation of protein from liver, plasma, and muscle extracts, followed by acid hydrolysis, and analysis for distribution of radioactivity among the various amino acids demonstrated that 94 to 99% of the radioactivity incorporated into protein was tyrosine. These data demonstrate incorporation of labeled intact tyrosine into protein. Labeling did not result from conversion of tyrosine into nonessential amino acids with subsequent incorporation of nonessential amino acids into protein.

DISCUSSION

The evidence for metabolic utilization of tyrosine when administered as N-Ac-Tyr consists of three elements. First, 25 to 36% of the radioactivity administered appears as ^{14}C -carbon dioxide when N-Ac-Tyr was administered as part of the parenteral regimen at either 0.5 or 2.0 mmol/kg body weight per day. This clearly indicates hydrolysis of N-Ac-Tyr and oxidation of the tyrosine. Second, the increased plasma tyrosine levels noted during infusion of N-Ac-Tyr at 2.0 mmol/kg body weight per day, and the presence of radioactivity as tyrosine in the free amino acid pools of both plasma and tissues indicates its availability. Finally, and most importantly from the perspective of growth and tissue repair, radioactive tyrosine was incorporated into both plasma and tissue proteins as tyrosine, and protein-bound tyrosine accounted for 80 to 90% of the radioactivity found in these tissues. The data demonstrate that tyrosine provided as N-Ac-Tyr is rapidly metabolized when administered as part of a parenteral alimentation solution and provides free tyrosine for energy substrate, tissue tyrosine pools, and protein synthesis.

However, utilization of N-Ac-Tyr as a parenteral tyrosine source appeared somewhat less than that observed with equimolar quantities of Ala-Tyr (8, 9). The percentage of infused labeled tyrosine oxidized to ^{14}C -carbon dioxide was lower for N-Ac-Tyr than for Ala-Tyr at both infusion levels (36.3 *versus* 42.4% at 0.5 mmol/kg body weight; 25.6 *versus* 54.1% at 2.0 mmol/kg body weight). Similarly, urinary losses were greater with N-Ac-Tyr than with Ala-Tyr (10.1 *versus* 7.7% at 0.5 mmol/kg body weight; 16.8 *versus* 5.4% at 2.0 mmol/kg body weight). These data indicate that N-Ac-Tyr is hydrolyzed more slowly by adult rats than Ala-Tyr.

Urinary tyrosine excretion was increased in animals infused with parenteral solutions containing N-Ac-Tyr when compared to values noted in animals infused with the same solution without added N-Ac-Tyr. This increased urinary loss of tyrosine was not due to an "overflow" aminoaciduria in animals infused at 0.5 mmol/kg body weight of N-Ac-Tyr per day because plasma tyrosine levels did not differ from those noted in control animals infused without N-Ac-Tyr. The increased urinary losses of tyrosine noted in animals infused at 2 mmol N-Ac-Tyr/kg body weight per day may reflect the increased plasma tyrosine levels observed in these animals. In general, the loss of urinary tyrosine in N-Ac-Tyr-infused animals was similar to that noted in animals infused with equimolar quantities of Ala-Tyr in earlier studies (8, 9).

Animals infused with N-Ac-Tyr lost greater quantities of the infused tyrosine source in urine than animals infused with Ala-Tyr. For example, in animals infused with Ala-Tyr at 0.5 mmol/kg body weight, 26% of the radioactivity excreted in urine was Ala-Tyr while 74% was tyrosine. Animals infused with 2.0 mmol/kg body weight Ala-Tyr lost 45% of the total radioactivity excreted as Ala-Tyr and 55% as free tyrosine. In contrast, animals infused with N-Ac-Tyr at 2 mmol/kg body weight per day excreted most of the label as intact N-Ac-Tyr (74% at 0.5 mmol/kg; 82% at 2.0 mmol/kg).

N-Ac-Tyr was not initially considered as a potential source of iv tyrosine in our studies, although it was soluble and easy to synthesize. We expected that its metabolism would be much less rapid than that of tyrosine peptides. Mammalian tissues contain N-acyl-amidohydrolases that catalyze the hydrolysis of N-acyl bonds of N-acetylated or N-formylated amino acids. The best described enzyme is acylase I, found in large amounts in the kidney (19–22). Although acylase I has good activity toward N-acyl-L-methionine and N-acyl-L-glutamate, it has little activity toward N-Ac-Tyr. Acylase II, a less well-described amidohydrolase (21, 23, 24), catalyzes the hydrolysis of N-acetyl-L-aspartate, but has little activity toward N-Ac-Tyr. Thus, the lack of reactivity of N-Ac-Tyr with these enzyme systems delayed our consideration of it as a tyrosine source. However, after our experiments with Ala-Tyr were completed, Endo (25) reported that mammalian tissues contain acylase III, which preferentially catalyzes the hydrolysis of the N-acyl derivatives of aromatic amino acids. Although the relative amounts of the three acylase enzymes in mammalian tissues have not been determined, our studies suggest sufficient acylase III activity for utilization of N-Ac-Tyr as a tyrosine source, at least in adult rats.

The studies outlined above indicate reasonable utilization of N-Ac-Tyr as a tyrosine source, but indicate that urinary excretion may limit utilization at higher infusion levels. It is important to recognize that our studies involved short-term infusion. It is possible that continued excretion of N-Ac-Tyr during long-term, high-dose infusion may produce some untoward metabolic effect not seen in short-term studies. At the present time a number of new amino acid solutions contain N-Ac-Tyr, but few utilization data in humans are available.

Snyderman (2) has presented evidence suggesting that tyrosine is an essential amino acid for some premature infants, even in the presence of adequate phenylalanine intake. Snyderman has estimated the tyrosine requirements for such orally fed infants to be 50 to 120 mg/kg/day (2, 26). Extrapolation of her oral feeding data to parenteral alimentation suggests the need for 50 to 120 mg (0.3 to 0.66 mmol) of tyrosine per 100 ml of solution, assuming an infusion rate of 100 to 120 ml/kg/24 h of standard parenteral solution of 2.5% amino acids–25% glucose. N-Ac-Tyr is very soluble and can be added to parenteral amino acid solutions in quantities sufficient to provide 2.5 mmol/100 ml of a 10% solution of amino acids. When such a solution is diluted with glucose to make the final 2% solution of amino acids, the final solution would contain 0.5 mmol/100 ml of N-Ac-Tyr. The solubility of N-Ac-Tyr would even permit development of the compound as an additive for iv solutions in a manner similar to that currently used for electrolytes.

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0031-3998/85/1906-0518\$02.00/0

PEDIATRIC RESEARCH

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Vol. 19, No. 6, 1985

Printed in U.S.A.

A Follow-Up Study of the Effects of Early Malnutrition on Subsequent Development. I. Physical Growth and Sexual Maturation during Adolescence

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ABSTRACT. A follow-up study of growth and development was conducted on 216 Barbadian children aged 9 to 15 yr, half of whom had histories of moderate to severe protein-energy malnutrition in their 1st yr of life. Although index girls had significant delays in sexual maturation and were reduced relative to comparison girls on measures of weight for height, arm circumference, and skinfold thick-

ness, their rate of growth when compared with values obtained 4 yr earlier was equal to or better than that of the comparison group. In contrast, boys in the index group were slightly reduced in height compared with their matched comparisons and had similar patterns of growth and sexual maturation. This study suggests a relationship between an episode of infantile malnutrition and impaired endocrine functioning among girls in the adolescent years. (*Pediatr Res* 19: 518-523, 1985)

Received July 9, 1984; accepted January 24, 1985.

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This work was performed in collaboration with the Ministries of Health and Education of Barbados and was supported by grants from the Thrasher Research Fund and the Ford Foundation.

Abbreviations

MANOVA, 2-way multivariate analysis of variance
ANOVA, 2-way univariate analysis of variance