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# Effects of Developmental Protein Malnutrition on Tryptophan Utilization in Brain and Peripheral Tissues[1]

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MILLER, M., J. P. LEAHY, F. MCCONVILLE, P. J. MORGANE AND O. RESNICK. *Effects of developmental protein malnutrition on tryptophan utilization in brain and peripheral tissues.* BRAIN RES. BULL. 2(5) 347–353, 1977. – Rats born of mothers fed a low protein diet (8% casein) compared to control rats on a normal diet (25% casein) started 5 weeks prior to mating showed significant decreases in the incorporation of parenterally injected $^{14}$C-tryptophan into protein in the brain and peripheral tissues. These effects were observed from day of birth to age 21 days, the oldest age assessed. Also, while different time-dependent patterns of uptake of labelled tryptophan were observed between the two diet groups at birth, this was not pronounced during the subsequent ages examined (Days 5–21). However, significant increases in uptake of $^{14}$C-tryptophan into brain and kidney of the 8% casein rats as compared to the 25% casein animals at ages 11 and 21 days may indicate the presence of a more active transport system for tryptophan in the malnourished animals. The significantly lower uptake, incorporation and percent incorporation of the tracer into liver of the 8% casein rats during ontogenetic development may indicate more rapid catabolism of liver proteins to maintain the concentrations of circulating amino acids. Overall, the data indicate that developmental protein malnutrition causes significant alterations in both brain and peripheral utilization of tryptophan.

Amino acid utilization  Tryptophan  Developmental protein malnutrition  Protein synthesis  Brain  Liver  Kidney

THE ROLE of the essential amino acid tryptophan in protein synthesis during development has been extensively investigated in recent years. These investigations have shown that tryptophan may have a limiting role in protein synthesis, especially in young animals [1, 2, 3, 11, 17]. As a result of these findings, attention has focused on the effects of pre- and postnatal malnutrition on endogenous tryptophan concentrations in the developing brain [3, 5, 10]. However, studies on the in vivo utilization of tracer amounts of tryptophan in the brains of prenatally protein malnourished rats during ontogenetic development have not been reported.

Because of the importance of tryptophan both in protein synthesis and also as a precursor for serotonin synthesis, alterations in its utilization under conditions of malnutrition could affect both of these metabolic pathways. For example, our group [14] and Sobotka *et al.* [13] have reported alterations in brain concentrations of serotonin in 21–22 day old rats as a consequence of pre- and postnatal protein malnutrition. We have also observed alterations in regional brain serotonin [10,14] and tryptophan [10] concentrations from birth to adulthood in rats which were protein malnourished. In the present report two studies were performed examining the utilization of peripherally injected $^{14}$C-tryptophan in regional brain areas and peripheral tissues in rats maintained on a normal diet or a protein deficient diet during the prenatal and postnatal development periods. The first study examined in detail the time course of $^{14}$C-tryptophan uptake and incorporation in protein on the day of birth, a time when the neurochemical effects of protein malnutrition are marked [8, 9, 10, 14, 15]. The second study compared the ontogenetic development of tryptophan utilization in protein synthesis from birth to age 21 days in normal and protein malnourished rats.

## METHOD

### *Animals*

Fourteen virgin albino Sprague-Dawley female rats, weighing 175–200 g, age 60–70 days (Charles River Laboratories, Inc.) were fed, ad lib, isocaloric diets (4.3 kcal/g) containing either a normal amount of protein (25% casein) or a low amount (8% casein). This dietary paradigm was started 5 weeks prior to mating (with a normal male) and continued through gestation and lactation. The composition of the diets is summarized elsewhere [4]. At birth, the litters were culled to 8 pups each and randomized across litters fed the same diet and born on the same day. Only litters containing the full complement of 8 pups were used for measurement of $^{14}$C-tryptophan uptake and protein

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TABLE 1

TIME COURSE OF $^{14}$C-TRYPTOPHAN UPTAKE AND INCORPORATION INTO PROTEIN IN THE BRAIN AND PERIPHERAL TISSUE OF NORMAL (25% CASEIN) AND PROTEIN MALNOURISHED (8% CASEIN) RAT PUPS ON THE DAY OF BIRTH. MEAN ± S.E.(N=5)

| | Minutes Between IP Injection of $^{14}$C-Tryptophan and Sacrifice | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10 | | 20 | | 90 | | 180 | |
| Diet | 8% | 25% | 8% | 25% | 8% | 25% | 8% | 25% |
| Homogenate (dpm X $10^2$/g) | | | | | | | | |
| telencephalon§ | 1031 ± 185 | 880 ± 102 | 1185 ± 56 | 1427 ± 170 | 3894 ± 489# | 2409 ± 260 | 2843 ± 139 | 3350 ± 328 |
| brainstem*§ | 1030 ± 205 | 1033 ± 104 | 1293 ± 96 | 1490 ± 139 | 3256 ± 190# | 2401 ± 303 | 2643 ± 88# | 2936 ± 73 |
| liver | 2835 ± 490 | 2957 ± 362 | 2450 ± 291 | 2676 ± 287 | 7332 ± 500# | 5362 ± 492 | 5251 ± 374 | 6485 ± 485 |
| kidney§ | 2953 ± 399 | 3111 ± 239 | 3024 ± 413 | 3181 ± 395 | 7574 ± 596 | 7237 ± 842 | 5959 ± 295 | 6782 ± 284 |
| Protein Precipitate (dpm X $10^2$/g) | | | | | | | | |
| telencephalon§ | 34 ± 5# | 61 ± 10 | 52 ± 4¶ | 153 ± 19 | 1868 ± 271 | 1583 ± 158 | 1783 ± 95 | 2236 ± 273 |
| brainstem*§ | 46 ± 9# | 79 ± 11 | 61 ± 5¶ | 175 ± 17 | 1606 ± 82 | 1651 ± 216 | 1715 ± 38# | 1947 ± 41 |
| liver§ | 204 ± 31# | 635 ± 118 | 291 ± 15¶ | 968 ± 135 | 5109 ± 347# | 4010 ± 377 | 3709 ± 257# | 4808 ± 366 |
| kidney+§ | 142 ± 21# | 315 ± 34 | 239 ± 41# | 705 ± 173 | 4686 ± 414 | 4944 ± 703 | 3875 ± 212# | 4863 ± 263 |
| Percent of Radioactivity in Protein Precipitate | | | | | | | | |
| telencephalon†§ | 3 ± 0¶ | 7 ± 1 | 4 ± 0¶ | 11 ± 1 | 48 ± 3¶ | 66 ± 1 | 63 ± 1# | 67 ± 1 |
| brainstem*†§ | 4 ± 0¶ | 8 ± 0 | 5 ± 0¶ | 12 ± 1 | 50 ± 3¶ | 69 ± 2 | 65 ± 2 | 66 ± 1 |
| liver†§ | 7 ± 0¶ | 21 ± 2 | 12 ± 2¶ | 36 ± 1 | 70 ± 3 | 75 ± 1 | 71 ± 2 | 74 ± 1 |
| kidney†§ | 5 ± 0¶ | 10 ± 1 | 8 ± 1¶ | 21 ± 2 | 62 ± 1# | 67 ± 2 | 65 ± 1# | 72 ± 2 |

*Consists of diencephalon, midbrain, pons-medulla and cerebellum.
†Significant ($p<0.001$) ⌐ differences between 8% and 25% groups for this row (analysis of variance).
*Significant ($p<0.05$) ⌙
§Significant ($p<0.001$) effects due to time for this row (analysis of variance).
¶$p<0.001$ ⌐
#$p<0.05$ ⌙ compared to 25% controls, post hoc t-tests (2-tailed).

synthesis at ages 0 (birth), 5, 11 and 21 (prior to weaning) days.

*Procedure*

*Injection, sacrifice and brain dissection.* L-[3-$^{14}$C]-tryptophan (specific activity 56.8 mCi/mM, New England Nuclear) dissolved in 0.9% saline was injected in a volume of 0.05–0.10 ml, IP, into rat pups of either sex at a dose of 10 $\mu$Ci/100 g. The pups were then returned to their dams until the time of sacrifice. In the time course experiment of protein synthesis on day of birth (Study 1), the intervals between $^{14}$C-tryptophan injection and sacrifice by guillotine were 10, 20, 90 and 180 min (n = 5 per group). In the ontogenetic experiment (Study 2) for ages 0 (birth), 5, 11 and 21 days (n = 5 per group) the interval between $^{14}$C-tryptophan injection and sacrifice was 20 min. At ages 0, 5 and 11 days the brain was dissected into the telencephalon and brainstem by making a cut through the thalamic projections to the cortex. At 21 days the brain was dissected into the telencephalon, diencephalon, midbrain, pons-medulla, and cerebellum according to previously described procedures [14]. Liver and kidney samples were also taken at all ages in order to assess the specificity of the changes observed in the brains of the normal and malnourished rats. Tissue samples were frozen in liquid nitrogen and stored at −20°C until analysis.

*Tissue processing and liquid scintillation counting.* The tissue samples were homogenized in cold 5% trichloracetic acid (TCA). An aliquot of the homogenate was taken to determine total radioactivity and the remainder was centrifuged at 1000 g for 10 min. Then, a sample of the supernate was taken to determine the radioactivity of the aqueous fraction. The precipitate, consisting primarily of protein but also containing proteolipids and lipids, was washed twice with cold 5% TCA and dissolved in NCS (Amersham/Searle) as were the supernate and homogenate samples. The NCS-tissue solutions were added to toluene scintillation solution containing 4 g PPO and 50 mg POPOP/1. Disintegrations per minute (dpm) were determined using channels-ratio method on a Packard Scintillation Spectrometer with a $^{14}$C counting efficiency of 70–75%. The results are expressed as dpm/g for homogenates and precipitates and as the percentage of tissue radioactivity present in each precipitate. This percentage primarily represents the proportion of $^{14}$C-tryptophan present in each tissue which is incorporated into a TCA-precipitable protein.

## RESULTS

*Study 1 (Day of Birth)*

The time course from 10–180 min of the uptake and incorporation of $^{14}$C-tryptophan on the day of birth is shown in Table 1. The results were evaluated by a 2-way analysis of variance (diet by time) for each row in Table 1. When significant diet by time interactions occurred, post hoc *t*-test of 8 vs. 25% values were made. To determine whether significant time-related differences occurred in the uptake and incorporation of radioactivity for either diet group, intra-group post hoc 2-tailed *t*-tests were also performed when a significant time effect was found.

The uptake of $^{14}$C-tryptophan into brain homogenates on the day of birth showed no significant differences between the two diet groups for the first two time points

investigated, i.e., 10 and 20 min. At 90 min, however, the malnourished pups showed significant increases of 35–60% in uptake of radioactivity into brain as compared to the control rats. The incorporation of radioactivity into TCA-precipitates of the brain samples showed significant differences between the two diet groups. The malnourished rats were utilizing 45–65% less $^{14}$C-tryptophan into the protein of the telencephalon at 10 and 20 min, and 10–65% less into the brainstem at 10, 20 and 180 min as compared to the 25% controls. Also, the percent incorporation of total tissue radioactivity into TCA-precipitates of the brain showed that the malnourished rats had significant decreases as compared to the normal pups. These decreases were 6–60% for the telencephalon at all four time points investigated, and 25–55% for the brainstem at 10, 20 and 90 min.

In the periphery, the uptake of $^{14}$C-tryptophan into homogenates of the liver and kidney showed no significant differences between the malnourished and normal pups at most time points investigated. The single exception was seen for the liver homogenates at 90 min, with the malnourished pups having significantly greater uptake of tracer as compared to the normal animals. Significant decreases in incorporation of $^{14}$C-tryptophan into TCA-precipitates in both the liver and kidney samples of the 8% pups were noted at 10, 20 and 180 min as compared to the control rats. These decreases were 20–70% for liver incorporation, and 20–65% for kidney incorporation. The percent incorporation of total radioactivity for liver showed that the malnourished pups were 65% lower at 10 and 20 min as compared to the normal rats. In the kidney the decrease in percent incorporation for the malnourished animals was 10–60% lower at all time points investigated as compared to the control rats.

The intra-group analysis of $^{14}$C-tryptophan utilization by the brain on the day of birth showed significant differences between the normal and malnourished rats. The malnourished pups reached a maximum uptake of tracer into the brain at 90 min ($p<0.001$), which was then followed by a significant decline in uptake between 90–180 min ($p<0.02$). The control rats, however, showed a significant uptake of tracer into the telencephalon across the time interval from 10–180 min ($p<0.05$ to $p<0.01$). The incorporation of radioactivity into TCA-precipitates of the brainstem showed that both groups reached a maximum at 90 min which then remained constant from 90–180 min. In the telencephalon, however, the 8% pups reached a peak incorporation at 90 min ($p<0.001$) which remained constant between 90–180 min, whereas, the 25% rats showed significant increases in incorporation across the time interval from 10–180 min ($p<0.05$ to $p<0.001$). The percent incorporation of total radioactivity into the brain showed that the control pups reached a maximum at 90 min for both brain samples ($p<0.001$), whereas, the malnourished pups showed significant increases in the time interval from 90–180 min for both brain samples ($p<0.01$).

In the periphery, the intra-group analysis of the utilization of $^{14}$C-tryptophan also showed significant differences between the two diet groups. For both liver and kidney homogenates, the malnourished and normal rats showed maxima at 90 min ($p<0.001$). However, the 8% rats showed a significant decrease in uptake of tracer for these tissues between 90–180 min ($p<0.05$), whereas, the 25% group showed a constant uptake pattern for this time interval. A similar pattern was observed for the incorporation of tracer into TCA-precipitates of the liver. The malnourished pups showed a significant decrease in incorporation of tracer between 90–180 min ($p<0.01$), while the control animals showed a constant incorporation during this time period. In the kidney, no significant differences for incorporation of radioactivity were noted between the two diet groups. Also, the percent incorporation of total radioactivity into TCA-precipitates of the liver showed no significant differences between the malnourished and normal rats. However, in the kidney the 8% pups showed significant increases in percent incorporation of $^{14}$C-tryptophan from 10–180 min ($p<0.01$), while the 25% rats showed a maximum at 90 min ($p<0.001$) which remained constant to 180 min.

*Study 2 (Birth–21 Days)*

The ontogeny of the brain and body weight is given in Table 2. Both the brain and body weights were equal in the normal and low protein groups on Days 0 and 5. Starting on Day 11 the body weights of the malnourished rats were significantly less ($p<0.001$) than the controls. By age 21 days the body weights of the malnourished animals were about 40% less than that of the normal rats. However, no significant differences were seen between the two diet groups for brain weights on Days 11 and 21.

The uptake and incorporation of $^{14}$C-tryptophan into the brain and peripheral tissues as a function of age are given in Table 2. The results were evaluated by a 2-way analysis of variance (diet by age) and significance levels for individual diet comparisons are indicated in Table 2. These results show that the malnourished rats had significant increases of 20–30% in uptake of tracer into homogenates of the brain at 11 and 21 days as compared to the control rats. The incorporation of the tracer into TCA-precipitates of the brains showed significant differences between the two diet groups on Days 0 and 5, with the malnourished rats showing a significant decrease of 50–65% for incorporation of radioactivity into the telencephalon and brainstem as compared to the control rats. However, no significant differences between the two diet groups were seen in the amount of incorporation of the tracer into brain on Days 11 and 21. The percent incorporation of total radioactivity into protein showed that the malnourished rats were significantly lower than the controls from Days 0–21. These decreases were 20–60% in the telencephalon, and 25–55% for the brainstem.

In the periphery, the liver showed significant differences in uptake of $^{14}$C-tryptophan into homogenates on Days 5 and 21. At these ages the malnourished rats showed significant decreases of 15–25% in uptake of tracer as compared to the control animals. In the kidney, however, the 8% animals showed significant increases of about 15% in uptake of tracer on Days 11 and 21 as compared to the 25% rats. The incorporation of radioactivity into TCA-precipitates for both liver and kidney showed that the malnourished rats were utilizing significantly less dpm/g in these tissues than the normal animals. On Days 0, 5 and 21 the decreases in incorporation for liver was 30–70%, and on Days 0 and 5 the decrease in incorporation seen in the kidney was 40–65%. Also, the percent incorporation of total radioactivity into protein for these tissues showed that the malnourished rats were significantly lower than the control rats at all ages examined. These decreases were 15–65% for the liver, and 15–60% for the kidney.

The intra-diet analysis for brain tissues showed that both diet groups reached a maximum uptake of $^{14}$C-tryptophan

TABLE 2

ONTOGENY OF WEIGHT GAIN AND OF $^{14}$C-TRYPTOPHAN UPTAKE AND INCORPORATION INTO PROTEIN IN BRAIN AND PERIPHERAL TISSUES OF NORMAL (25% CASEIN) AND PROTEIN MALNOURISHED (8% CASEIN) RAT PUPS. MEAN ± S.E. (N=5)

| Days of Age | 0 (Birth) | | 5 | | 11 | | 21 | |
|---|---|---|---|---|---|---|---|---|
| Diet (% Casein) | 8% | 25% | 8% | 25% | 8% | 25% | 8% | 25% |
| Body weight (g) | 6.1 ± 0.1 | 6.1 ± 0.1 | 11.3 ± 0.5 | 13.4 ± 1 | 15.9 ± 0.6¶ | 29.5 ± 0.8 | 35.2 ± 0.4¶ | 60.8 ± 1.9 |
| Brain weight (mg) | 226 ± 6 | 212 ± 6 | 488 ± 35 | 420 ± 22 | 982 ± 29 | 1030 ± 16 | 1428 ± 35 | 1512 ± 56 |
| Homogenate (dpm X $10^2$/g) | | | | | | | | |
| telencephalon§ | 1185 ± 56 | 1427 ± 170 | 629 ± 28# | 819 ± 59 | 1439 ± 146# | 1062 ± 26 | 1303 ± 63# | 1026 ± 91 |
| brainstem*§ | 1293 ± 96 | 1490 ± 139 | 756 ± 27# | 851 ± 29 | 1437 ± 158# | 1059 ± 34 | 1337 ± 62# | 1044 ± 72 |
| liver+§ | 2450 ± 291 | 2676 ± 287 | 1788 ± 126# | 2305 ± 103 | 3142 ± 520 | 3601 ± 109 | 4213 ± 238# | 5078 ± 143 |
| kidney§ | 3024 ± 413 | 3181 ± 395 | 2313 ± 200 | 2417 ± 205 | 4481 ± 254# | 3722 ± 222 | 5276 ± 183# | 4510 ± 210 |
| Protein Precipitate (dpm X $10^2$/g) | | | | | | | | |
| telencephalon+§ | 52 ± 4¶ | 153 ± 19 | 129 ± 13¶ | 308 ± 21 | 358 ± 48 | 433 ± 21 | 465 ± 25 | 481 ± 61 |
| brainstem*+§ | 60 ± 5¶ | 176 ± 17 | 147 ± 11¶ | 292 ± 9 | 369 ± 57 | 433 ± 23 | 510 ± 28 | 542 ± 47 |
| liver+§ | 291 ± 15¶ | 968 ± 135 | 774 ± 84¶ | 1318 ± 61 | 1515 ± 319 | 1982 ± 69 | 2371 ± 122¶ | 3386 ± 76 |
| kidney†§ | 239 ± 41# | 705 ± 173 | 581 ± 79# | 992 ± 66 | 1176 ± 148 | 1269 ± 98 | 1932 ± 98 | 2039 ± 97 |
| Percent of Radioactivity in Protein Precipitate | | | | | | | | |
| telencephalon†§ | 4 ± 0¶ | 11 ± 1 | 21 ± 1¶ | 38 ± 1 | 25 ± 1¶ | 41 ± 1 | 36 ± 3# | 46 ± 2 |
| brainstem*†§ | 5 ± 0¶ | 12 ± 1 | 20 ± 2¶ | 35 ± 2 | 25 ± 3¶ | 41 ± 1 | 38 ± 3# | 52 ± 1 |
| liver†§ | 12 ± 2¶ | 36 ± 1 | 43 ± 2¶ | 57 ± 1 | 47 ± 2¶ | 55 ± 1 | 56 ± 2¶ | 67 ± 1 |
| kidney†§ | 8 ± 1¶ | 21 ± 2 | 25 ± 2¶ | 41 ± 2 | 26 ± 2¶ | 34 ± 1 | 37 ± 3# | 45 ± 0 |

*Consists of diencephalon, midbrain, pons-medulla and cerebellum.
†Significant ($p<0.001$) ┐differences between 8% and 25% groups for this row (analysis of variance).
+Significant ($p<0.05$) ┘
§Significant ($p<0.001$) effects due to age for this row (analysis of variance).
¶$p<0.001$ ┐compared to 25% controls, post hoc t-tests (2-tailed).
#$p<0.05$ ┘

into homogenates at Day 11 which then remained constant to age 21 days. Also, the incorporation of tracer into protein showed that both diet groups reached a maximum incorporation in the telencephalon at Day 11 which then remained constant to Day 21. In the brainstem significant increases in incorporation were seen from Days 0–21 for both groups. However, the percent incorporation of total radioactivity showed significant differences between the two groups. The malnourished rats showed significant increases into telencephalon from Days 0–21 ($p<0.01$), whereas, the 25% rats showed a maximum in this brain area at Day 5 ($p<0.001$) which remained constant to Day 21. In the brainstem, the malnourished rats showed maxima on Days 5 and 21, while the control rats showed significant increases from Days 0–21 ($p<0.02$ to $p<0.001$).

The uptake and incorporation of $^{14}$C-tryptophan into peripheral tissues by intra-diet analysis showed no significant ontogenic differences between the two diet groups for uptake of tracer into kidney homogenates. In the liver, however, the malnourished animals showed a maximum on Day 11 ($p<0.05$) which remained constant to Day 21, while the control rats showed maxima for uptake in this tissue on Days 11 and 21 ($p<0.001$). The incorporation of tracer into protein showed no significant differences between the two diet groups for the liver. In the kidney, however, significant differences were seen for the incorporation of $^{14}$C-tryptophan. The malnourished rats showed significant increases in incorporation from Days 0–21 ($p<0.01$), whereas, the control rats showed a maximum on Day 11 ($p<0.05$) which remained constant to Day 21. The percent incorporation of total radioactivity into protein showed no significant differences between the 8% and 25% rats for liver and kidney. Both diet groups showed maxima on Days 5 and 21.

Table 3 provides a more detailed regional brain distribution of $^{14}$C-tryptophan in the 21 day normal and malnourished rats. The uptake of radioactivity into tissue homogenates showed that in all areas examined the 8% rats had significant increases of 20–25% as compared to the control animals. However, the incorporation of $^{14}$C-tryptophan into TCA-precipitates showed no significant differences between the two diet groups. The percent incorporation of total tissue radioactivity into protein showed that in all areas examined the malnourished rats had significant decreases of 20–30% as compared to the normal animals.

## DISCUSSION

The results presented here clearly demonstrate that developmental protein malnutrition effects the uptake and incorporation of $^{14}$C-tryptophan into brain and peripheral tissues. Significant alterations in the systemic utilization of the tracer were seen from birth to age 21 days in the malnourished rats. At birth, these animals exhibited significant decreases in the amount of incorporation and in the percent incorporation of total radioactivity into brain and peripheral tissues as compared to the control rats. The altered utilization of the tracer by the normal and

TABLE 3

REGIONAL BRAIN DISTRIBUTION OF RADIOACTIVITY FOLLOWING IP INJECTION OF $^{14}$C-TRYPTOPHAN IN 21 DAY OLD NORMAL AND PROTEIN MALNOURISHED RATS. MEAN ± S.E.(N=5)

| | Homogenate dpm X $10^2$/g | | TCA-Precipitate dpm X $10^2$/g | | Percent of Tissue Radioactivity in Precipitate | |
|---|---|---|---|---|---|---|
| Diet (% Casein) | 8% | 25% | 8% | 25% | 8% | 25% |
| Telencephalon | 1303 ± 63† | 1026 ± 91 | 465 ± 25 | 481 ± 61 | 36 ± 3† | 46 ± 2 |
| Diencephalon | 1262 ± 78† | 966 ± 57 | 450 ± 36 | 466 ± 43 | 36 ± 3† | 48 ± 2 |
| Midbrain | 1304 ± 86* | 1028 ± 65 | 497 ± 29 | 499 ± 35 | 39 ± 3† | 48 ± 1 |
| Cerebellum | 1390 ± 71* | 1118 ± 95 | 533 ± 36 | 594 ± 63 | 38 ± 3* | 53 ± 2 |
| Pons-Medulla | 1368 ± 59† | 1049 ± 92 | 551 ± 36 | 588 ± 53 | 41 ± 5† | 56 ± 2 |

*p<0.001
†p<0.02
*p<0.05, compared to 25% values, 2-tailed t-tests.

malnourished pups at birth cannot be attributed to differences in brain and body weight since, at this age, these parameters were identical for the two diet groups. Also, the two diet groups displayed different utilization patterns over the 180 min time course investigated. The malnourished pups showed a peak in their uptake of tracer into brain and peripheral tissues at 90 min, which was followed by a significant decrease in uptake of $^{14}$C-tryptophan into all tissues examined in the interval between 90–180 min. The normal pups, on the other hand, showed a constant uptake into brain and peripheral tissues in this time interval. While both groups showed essentially the same pattern for incorporation of tracer into brain and peripheral tissues, significant differences were seen between the normal and malnourished rats for percent incorporation of total radioactivity into protein. The control group reached a maximum percent incorporation at 90 min into brain and peripheral tissues which then remained constant to 180 min. This pattern was also seen for the liver of the malnourished pups. However, in the brain and kidney the malnourished rats showed significant increases in percent incorporation of tracer across the time interval from 10–180 min.

The decrease in incorporation and percent incorporation of $^{14}$C-tryptophan into brain and peripheral protein in the malnourished pups on the day of birth was in contrast to our findings in the case of $^{14}$C-phenylalanine utilization [9]. In that study the malnourished rats showed significant increases in incorporation and percent incorporation of total radioactivity into both brain and peripheral tissues as compared to the control animals. Interestingly, one similarity was noted for both the $^{14}$C-phenylalanine study and these results on $^{14}$C-tryptophan utilization on day of birth. At 90 min in both of these tracer studies the malnourished pups showed a significant surge in uptake of radioactivity into both brain and peripheral homogenate as compared to the control animals. In the $^{14}$C-phenylalanine study this surge was then followed by a steady uptake of tracer into brain and liver and a significant decrease in kidney uptake. In the present investigation, the 90 min surge was followed by significant decreases in uptake of $^{14}$C-tryptophan for all tissues examined. The reason(s) for the 90 min surge remains unclear. Perhaps it represents a more active transport system for amino acids which is then followed by a faster catabolism of amino acids in the malnourished animals.

The effects of diet on the uptake and incorporation of $^{14}$C-tryptophan into brain and peripheral tissues on subsequent Days 5, 11 and 21 showed significant differences between the normal and protein malnourished animals. The malnourished rats had significant increases as compared to the normal animals in uptake of the tracer into brain and kidney tissues on Days 11 and 21. However, the most notable effect of the low protein diet was seen in the incorporation and percent incorporation of $^{14}$C-tryptophan into both brain and peripheral protein. The malnourished rats showed significant decreases in brain and kidney incorporation of tracer on Days 0 and 5, and percent incorporation into protein into these tissues from Days 0–21 as compared to the control animals. For liver, significant decreases in incorporation were noted on Days 0, 5 and 21 and for percent incorporation of tracer on Days 0–21.

Group patterns of uptake and incorporation of radioactivity during ontogenetic development displayed some differences between the two diet groups. Both groups reached a peak uptake of $^{14}$C-tryptophan into the brain on Day 11 and in kidney on Day 21. However, in the liver the malnourished rats had a maximum uptake on Day 11, whereas, the control animals showed a maximum on Day 21. Similar patterns were also noted for incorporation and percent incorporation of tracer into brainstem and liver for the two diet groups with maxima observed on Day 21. While both diet groups showed peak incorporation into the telencephalon on Day 11, the malnourished rats had a maximum percent incorporation of tracer on Day 21, whereas, the controls showed a peak on Day 11. In the kidney, the control rats showed maximum incorporation on Day 11, however, the malnourished animals exhibited a peak on Day 21. Both diet groups showed maximum percent incorporation of tracer into kidney on Day 21.

The lowered systemic incorporation of $^{14}$C-tryptophan into protein seen in the malnourished rats as a consequence of developmental protein malnutrition had been postulated earlier [9]. We proposed that the high endogenous concentrations of free plasma and brain tryptophan at birth [8,10], and at older ages [10], in pre- and postnatally protein deprived rats would cause a lower incorporation of

exogenous labelled tryptophan into brain and peripheral protein. At birth, the malnourished pups had brain and free plasma tryptophan concentrations that were approximately twice those of the normal pups [8,10]. In the present study we observed significant decreases of up to 50–70% for incorporation of $^{14}$C-tryptophan into protein in brain and peripheral tissues of the malnourished pups as compared to the control rats at birth. The lowered incorporation of tracer into proteins at this age can probably be accounted for by two characteristics of the malnourished rats: (1) dilution of the tracer by the high endogenous systemic tryptophan concentrations and; (2) the lowered affinity of tryptophan for the incorporation system as reported by Oja [12]. However, there are no indications that the malnourished pups were converting excessive amounts of tryptophan to serotonin at the expense of protein synthesis. We have reported significant elevations in brain [10,14], and peripheral [14] serotonin concentrations in malnourished rats on day of birth. The serotonin concentrations for both diet groups in those studies indicate, however, that less than 5% of their endogenous tryptophan was being utilized in serotonin synthesis. Interestingly, both diet groups incorporated no more than 65–70% of the total $^{14}$C-tryptophan into brain and peripheral protein. The remaining 30% of the tracer is probably being converted into indolepyruvic acid by tryptophan aminotransferase rather than to serotonin by tryptophan hydroxylase. This interpretation is based on the observation that the activity of the former enzyme [6] is comparatively much higher than the latter [16] in brain and peripheral tissues of newborn rats.

The decreased incorporation of $^{14}$C-tryptophan into brain and peripheral tissues of the malnourished rats during ontogenetic development may similarly be the consequence of a lower affinity for the incorporation system due to their higher endogenous tryptophan concentrations. While the brain and free plasma tryptophan concentrations of the malnourished rats on Days 5–21 were considerably lower than their birth values, they were significantly greater than the control rats at these ages [10]. However, the malnourished rats showed significant increases in uptake of $^{14}$C-tryptophan into brain and kidney on Days 11 and 21 as compared to the normal rats. Since the absolute percent increase of uptake of tracer into the kidney was less than that seen in the brain, this may imply the presence of a more active transport system for essential amino acids into the brains of the malnourished animals. This phenomena was also noted in our study on $^{14}$C-phenylalanine uptake into the brains of protein malnourished rats during ontogenetic development [9] and may represent a form of brain-sparing. This sparing may also be reflected in the nonsignificant differences in brain weights between the two diet groups as compared to the significantly greater differences in body weights as compared to a 5% difference in brain weights between the malnourished and normal rats.

The significant decreases in uptake and incorporation of $^{14}$C-tryptophan into the liver of the malnourished rats during ontogenetic development may indicate that the concentrations of circulating tryptophan and possibly other amino acids are being maintained by some catabolism of liver proteins. We have reported significant decreases in plasma albumin concentrations in protein deprived rats during ontogenetic development [10]. Since albumin manufacture occurs only in the liver, the lower circulating plasma albumin concentrations seen in these animals may indicate less synthesis in liver due to a higher catabolism of liver proteins. The decreased albumin concentrations seen in the malnourished rats is one of the reasons for their higher endogenous tissue tryptophan concentrations. Since tryptophan is the only amino acid found bound to plasma albumin [7], the lower amounts of albumin seen in the malnourished rats causes them to have a greater amount of free plasma tryptophan available for transport to tissues. Therefore, while these animals have more tryptophan available for transport to tissues, the lowered affinity of tryptophan for the incorporation system causes a lower incorporation of $^{14}$C-tryptophan into protein.

In conclusion, the present results demonstrate that the alterations in tryptophan availability due to pre- and postnatal malnutrition in the pregnant rat are correlated with significant decreases in the incorporation of $^{14}$C-tryptophan into protein by the pups from birth to age 21 days. This lowered incorporation can be directly related to the lowered affinity (or dilution) of the tracer by the significantly higher endogenous concentrations of brain and free plasma tryptophan reported for the malnourished animals. However, the altered systemic tryptophan concentrations seen in the malnourished rat may represent the consequence of a need to ensure the availability of tryptophan for protein synthesis and development in early life.

## REFERENCES


1. Allen, R. E., P. L. Raines and D. M. Regen. Regulatory significance of transfer RNA charging levels 1. Measurements of charging levels in livers of chow-fed rats, fasting rats, and rats fed balanced or imbalanced mixtures of amino acids. *Biochim. Biophys. Acta* **190:** 323–336, 1969.
2. Aoki, K. and F. L. Siegel. Hyperphenylalaninemia: Disaggregation of brain polyribosomes in young rats. *Science* **168:** 129–130, 1970.
3. Dickerson, J. W. T. and S.-K. Pao. The effect of a low protein diet and exogenous insulin on brain tryptophan and its metabolites in the weanling rat. *J. Neurochem.* **25:** 559–564, 1975.
4. Forbes, W. B., O. Resnick, W. C. Stern, J. D. Bronzino and P. J. Morgane. The effect of chronic protein malnutrition on trans-callosal evoked responses in the rat. *Devl Psychobiol.* **8:** 503–509, 1975.
5. Kalyanasundaram, S. Effect of dietary protein and calorie deficiency on tryptophan levels in the developing rat brain. *J. Neurochem.* **27:** 1245–1247, 1976.
6. Lolova, I. and A. Dikow. Histochemical evidence of aminotransferases VI. Quantitative histochemical and biochemical investigation of aspartate-, alanine- and aromatic aminotransferases in the rat organs during development. *Acta Histochem.* **55:** 225–240, 1976.
7. McMenamy, R. H. and J. L. Oncley. The specific binding of L-tryptophan to serum albumin. *J. Biol. Chem.* **233:** 1436–1447, 1958.
8. Miller, M., O. Resnick, J. P. Leahy and P. J. Morgane. Brain and peripheral tryptophan availability in protein malnourished rats. Society for Neuroscience (6th Annual Meeting), Toronto, Canada, p. 586, 1976.

9. Miller, M., J. P. Leahy, F. McConville, P. J. Morgane and O. Resnick. Phenylalanine utilization in brain and peripheral tissues during development in normal and protein malnourished rats. *Brain Res. Bull.* **2**: 189–195, 1977.
10. Miller, M., J. P. Leahy, W. C. Stern, P. J. Morgane and O. Resnick. Tryptophan availability: Relation to elevated brain serotonin in developmentally protein-malnourished rats. *Expl Neurol.* **57**: 142–157, 1977.
11. Nakano, L., T. Kishi, N. Kurita and K. Ashida. Effect of dietary amino acids in amino acid-catabolizing enzymes in rat liver. *J. Nutr.* **100**: 827–836, 1970.
12. Oja, S. S. Incorporation of phenylalanine, tyrosine and tryptophan into protein of homogenates from developing rat brain: Kinetics of incorporation and reciprocal inhibition. *J. Neurochem.* **19**: 2057–2069, 1972.
13. Sobotka, T. J., M. P. Cook and R. E. Brodie. Neonatal malnutrition: Neurochemical, hormonal and behavioral manifestations. *Brain Res.* **65**: 443–457, 1974.
14. Stern, W. C., M. Miller, W. B. Forbes, P. J. Morgane and O. Resnick. Ontogeny of the levels of biogenic amines in various parts of the brain and in peripheral tissues in normal and protein malnourished rats. *Expl Neurol.* **49**: 314–326, 1975.
15. Stern, W. C., M. Miller, W. B. Forbes, J. P. Leahy, P. J. Morgane and O. Resnick. Effects of protein malnutrition during development on protein synthesis in brain and peripheral tissues. *Brain Res. Bull.* **1**: 27–31, 1976.
16. Wapnir, R. A., R. L. Hawkins and J. H. Stevenson. Ontogenesis of phenylalanine and tryptophan hydroxylation in rat brain and liver. *Biol. Neonate* **18**: 85–93, 1971.
17. Wunner, W. H., J. Bell and H. Munro. The effect of feeding with a tryptophan-free amino acid mixture on rat-liver polysomes and ribosomal ribonucleic acid. *Biochem. J.* **101**: 417–428, 1966.