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Tryptophan Availability: Relation to Elevated Brain Serotonin in Developmentally Protein-Malnourished Rats

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Developmental changes in tryptophan, serotonin, and 5-hydroxyindoleacetic acid in many brain regions were examined in normal and protein-malnourished rats from birth to age 30 days. The malnourished rats, whose dams received a diet low in protein starting 5 weeks prior to conception, showed significantly elevated brain tryptophan, serotonin, and 5-hydroxyindoleacetic acid at most ages examined. Brain tryptophan concentrations for both groups of animals showed a positive correlation with their respective unbound plasma tryptophan concentrations. Although the malnourished animals showed lower total plasma tryptophan concentrations than the control group, the amount of free plasma tryptophan available for brain metabolism was significantly higher in the malnourished rats. This was due, in part, to a decrease in the molar ratio of bound tryptophan to albumin in the malnourished animals. In addition, those malnourished rats had lower albumin levels and higher concentrations of nonesterified fatty acids as compared to the normal animals, causing more tryptophan to be available as the free form in plasma. Overall, the present results demonstrate that rearing rats on a diet low in protein but adequate in all other respects significantly elevates brain tryptophan and amine concentrations, probably as a consequence of developmental alterations in plasma tryptophan availability.

INTRODUCTION

The vulnerability of the developing central nervous system to insults of protein or protein–calorie malnutrition has been extensively investigated in recent years. Various studies have demonstrated alterations in the DNA content (14), and the morphological (14, 26), physiological (25, 27), and

Abbreviations: NEFA—nonesterified fatty acids, 5-HIAA—5-hydroxyindoleacetic acid.

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biochemical (1, 28) properties in rats, as a result of postnatal malnutrition. Little attention has been given to the effects of prenatal malnutrition, because it has been thought that the most serious effects of undernutrition on the central nervous system occur during the so-called postnatal brain-growth spurt period of rapid development (13). Several recent studies have shown that changes occur in electrophysiological properties (6, 15, 33), behavior (30, 34, 36), and biogenic amine metabolism (29, 30, 34, 35) in rats, as a result of protein restriction to the dams started prior to conception and continued through lactation. Our group (35) reported elevated serotonin and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in both brain and peripheral tissues using this malnutrition paradigm, and suggested that general alterations in serotonin metabolism might be caused by alterations in tryptophan availability in the malnourished animals. In the present studies we investigated the effects of protein deficiencies started prior to gestation on the ontogeny of tryptophan, serotonin, and 5-hydroxyindoleacetic acid concentrations in local brain regions. To evaluate the possible relation between tryptophan concentrations in the plasma and its brain concentrations, tryptophan (both total and free), total protein, albumin, and nonesterified fatty acids (NEFA) concentrations were measured in plasma. The latter two factors were assessed because changes in their concentrations could cause alterations in the amount of free plasma tryptophan.

We examined the changes in plasma and brain tryptophan in protein-malnourished subjects because of the unique characteristics of this essential amino acid. It is the least abundant essential amino acid in nature and is the only amino acid which is bound to plasma albumin (21). Considerable evidence points to the fact that only the plasma tryptophan not bound to albumin is available for transport to tissue and for serotonin synthesis (5, 9, 19, 37). Also, free plasma tryptophan may be a limiting factor in protein synthesis, especially in young animals (2, 3, 12, 40). Therefore, by determining the effect of early protein malnutrition on tryptophan availability and utilization by the brain, we may better understand the neurochemical basis of altered central nervous system functioning seen in developmentally protein-malnourished rats.

METHODS

Dietary and Rearing Conditions. Virgin female albino Sprague-Dawley rats (Charles River Laboratories, Inc.) were fed isocaloric (4.3 kcal/g) diets containing either low protein (8% casein) or normal amounts (25% casein) for 5 weeks prior to mating with a normally fed male. A complete summary of the composition of the diets can be found in Forbes *et al.* (15). At birth each litter was culled to eight pups and randomized with other

litters fed the same diet and born the same day. After weaning at age 21 days, the rats were housed two to four per cage and continued receiving their respective diets until killed for chemical measures. Throughout the study all rats were given food and water *ad libitum* and maintained on a 12-h:12-h light:dark cycle (lights on at 0700 to 1900 h).

Chemical Procedures and Brain Dissection. The study used 96 rats of either sex. The subjects were taken from the nursery room to an adjoining laboratory for killing by guillotine between 1300 and 1500 h at ages 0 (birth), 5, 11, 16, 21 (weaning), and 30 days. After killing the rats, the brain tissues were quickly removed, frozen in liquid nitrogen, and stored at −20°C for subsequent analyses of serotonin, 5-HIAA, and tryptophan concentrations. The regional brain dissection procedure for the various age points was the same as previously described (35), with the maximum number of regions (at days 21 and 30) consisting of telencephalon, diencephalon, midbrain, cerebellum, and pons–medulla. Blood from the trunk of each rat was collected into individual heparinized tubes using no more than 0.1 to 0.2 mg heparin per milliliter whole blood to prevent the anticoagulant from increasing the concentration of free tryptophan in plasma (5).

Plasma was obtained by centrifugation of blood samples 10 min at 1000 *g*. Ultrafiltrate of plasma (to collect free tryptophan) was obtained using Amicon-Centriflo 224 CF-50 membrane cones. A volume of 0.15 to 0.6 ml plasma from each rat was placed into each cone and centrifuged 30 min at 800 *g*. Under those conditions, 50% to 75% of the initial volume of plasma passed through the dialysis membrane, i.e., the ultrafiltrate.

Serotonin and 5-hydroxyindoleacetic acid concentrations were measured in each brain sample by a modification of the spectrophotofluoremetric method of Maickel *et al.* (22) and Thompson et al. (39). Brain tryptophan was assayed on a portion of the serotonin-containing extract of each brain sample by the method of Denkla and Dewey (11). Total and free plasma tryptophan were measured on samples of plasma and ultrafiltrate, respectively, obtained from each rat by the same procedure used for brain tryptophan. Nonesterified fatty acids in each plasma sample were assayed using the procedure of Laurell and Tibbling (20). Albumin and total protein concentrations in all plasma samples were measured using General Diagnostics Albustrate and Hycell Biuret procedures, respectively.

Statistics. Evaluation of brain region data was by 36 two-way analyses of variance (anova) of diet by age (with repeated measures) for serotonin, 5-HIAA, and tryptophan at each of the six age points. When the diet–age interaction was significant, individual *post hoc t* test comparisons between the 8% and 25% groups were made. Evaluation of diet effects on plasma tryptophan, nonesterified fatty acids, albumin, and total protein were assessed by *t* tests at each age point.

RESULTS

At birth the average brain (286 to 299 mg) and body (6.1 to 6.4 g) weights were essentially the same in the animals fed 8% and 25% protein (bottom of Table 1). After birth, however, the brain and body growth profile of the two groups diverged, with the control group being significantly greater than the malnourished rats. These differences became more pronounced for body weight than for brain weight in the two groups. The normal rats first showed significantly greater weight gains than the malnourished animals for body weights at age 11 days and for brain weight at age 16 days. However, at age 30 days the malnourished rats had body and brain weights of about 30% and 90% of that of the controls, respectively.

The developmental changes in regional brain serotonin, 5-HIAA, and tryptophan concentrations are summarized in Table 1. The anova rows at the bottom of the amine, metabolite, and precursor portions of the table indicate the results of the overall diet factor comparisons. For most diet factor comparisons, the 8% rats had significantly higher serotonin, 5-HIAA, and tryptophan concentrations than the 25% rats. There was only one instance where the 25% group showed significantly higher levels than the 8% group. This was for brain tryptophan on day 16.

The results of comparisons of control group versus malnourished group values for individual brain regions are denoted in Table 1 by letter superscripts. The regional brain analyses for serotonin, 5-HIAA, and tryptophan showed that they were more highly concentrated in the diencephalon, midbrain, and pons–medulla regions in the malnourished rats than in the controls. It was these brain stem regions which showed the greatest increases in precursor, amine, and metabolite levels in the malnourished rats. However, the telencephalic region of the 8% rats also showed some elevations in these substances as compared to the 25% rats. Overall, these differences were most pronounced at age 0, 11, 21, and 30 days, with significant increases in levels in the 8% rats being 120% to 195% of the controls. Consequently, at these time points the whole brain values (calculated on a weighted basis of the parts) showed significant increases with the 8% group being 130% to 175% of the controls.

The ontogenetic changes in plasma tryptophan, nonesterified fatty acids, albumin, and total protein concentrations are given in Table 2, with letter superscripts denoting significant differences between the two diet groups. Changes in some of those parameters are also illustrated in Fig. 1. The total and free plasma tryptophan concentrations for the 8% and 25% animals (Table 2) showed that the control group had significantly elevated total tryptophan as compared to the malnourished group. On the average, the 25% values were 140% to 290% of the values for the 8% rats at most ages. The exceptions were on day 0, when the malnourished rats had

TABLE 1

Effects of a Low Protein Diet (8% Casein) on the

Age (days)	0 (Birth)		5		11	
Diets (*N*)	8% 8	25% 8	8% 8	25% 8	8% 8	25% 8
Brain region						
	Mean serotonin concentrations ± SE (ng/g)					
WB[a]	661 ± 21[b]	384 ± 29	381 ± 13[b]	311 ± 10	585 ± 11[b]	356 ± 17
Tel.	572 ± 32[b]	329 ± 32	245 ± 14	232 ± 12	357 ± 9[b]	246 ± 22
Dien.	—	—	—	—	1110 ± 45[b]	572 ± 23
Mid.	—	—	—	—	—	—
Cereb.	—	—	—	—	—	—
P–M[d,e]	783 ± 29[b]	462 ± 38	584 ± 22[b]	425 ± 31	838 ± 29[b]	502 ± 38
(Anova)	8% > 25%		8% > 25%		8% > 25%	
	Mean 5-hydroxyindoleacetic acid concentrations ± SE (ng/g)					
WB	926 ± 55[b]	546 ± 43	505 ± 32[b]	399 ± 15	710 ± 23[b]	425 ± 21
Tel.	789 ± 72[b]	524 ± 54	381 ± 34	315 ± 30	404 ± 22[b]	287 ± 17
Dien.	—	—	—	—	1301 ± 85[b]	673 ± 29
Mid.	—	—	—	—	—	—
Cereb.	—	—	—	—	—	—
P–M[d,e]	1113 ± 41[b]	574 ± 46	687 ± 39[b]	519 ± 26	1114 ± 45[b]	662 ± 42
(Anova)	8% > 25%		8% > 25%		8% > 25%	
	Mean tryptophan concentrations ± SE (ng/g)					
WB	15,694 ± 1173[b]	8785 ± 337	2315 ± 142[b]	1476 ± 105	3971 ± 271[b]	2747 ± 142
Tel.	15,398 ± 1213[b]	8508 ± 598	2330 ± 119[b]	1418 ± 83	3751 ± 344[b]	2405 ± 136
Dien.	—	—	—	—	4202 ± 177[b]	2711 ± 190
Mid.	—	—	—	—	—	—
Cereb.	—	—	—	—	—	—
P–M[d,e]	16,190 ± 1253[b]	9163 ± 425	2303 ± 240[c]	1565 ± 153	4342 ± 256[b]	3572 ± 328
(Anova)	8% > 25%		8% > 25%		8% > 25%	
Body weight (g)	6.1 ± 0.2	6.4 ± 0.2	10.4 ± 0.4[b]	14.8 ± 0.3	16.4 ± 0.2[b]	31.8 ± 1.9
Brain weight (mg)	299 ± 7	286 ± 11	666 ± 12	683 ± 17	1176 ± 28[b]	1361 ± 17

[a] Abbreviations: WB—whole brain; Tel.—telencephalon; Dien.—diencephalon; Mid.—midbrain; Cereb.—cerebellum; P–M—pons and medulla; Anova—outcome of diet factor in analysis of variance at each age; ns—no significant effect.

[b] $P < 0.001$.

[c] $P < 0.01$; two-tailed t tests.

[d] Consists of hypothalamus, midbrain, pons–medulla, and cerebellum at age 0 and 5 days.

[e] Consists of midbrain, pons–medulla, and cerebellum at age 11 and 16 days.

higher levels, and day 5 when there were no differences between the two diet groups. The 8% rats, however, showed significant elevations of free tryptophan concentrations as compared to the 25% rats on the order of 125% to 175% at all ages except day 16, when no differences between the two groups were observed. Also, the 8% group showed a significantly elevated percentage of free plasma tryptophan at all ages examined (Table 2).

The changes in the molar ratio of bound tryptophan to albumin are illustrated in Fig. 1a. Using a molecular weight of 67,000 for albumin, the concentrations given in Table 2 were converted to molar values. Bound tryptophan concentrations were calculated as the differences between the

Development of Brain Indole Metabolism and Growth

16		21		30	
8%	25%	8%	25%	8%	25%
8	8	8	8	8	8
		Mean serotonin concentrations ± SE (ng/g)			
422 ± 14	413 ± 22	511 ± 20[b]	379 ± 7	550 ± 14[b]	373 ± 6
294 ± 20	305 ± 31	331 ± 24[c]	266 ± 8	394 ± 20[b]	259 ± 8
794 ± 37	756 ± 33	870 ± 52	736 ± 51	910 ± 55[b]	606 ± 52
—	—	1051 ± 55[b]	770 ± 4	909 ± 46[b]	643 ± 29
—	—	352 ± 14[b]	229 ± 18	412 ± 19[b]	321 ± 15
505 ± 26	535 ± 26	1050 ± 28[b]	696 ± 23	949 ± 28[b]	728 ± 17
ns		8% > 25%		8% > 25%	
		Mean 5-hydroxyindoleacetic acid concentrations ± SE (ng/g)			
446 ± 22	415 ± 24	650 ± 16[b]	462 ± 7	662 ± 25[b]	430 ± 8
309 ± 28	297 ± 32	416 ± 21[b]	289 ± 12	454 ± 31[b]	250 ± 14
801 ± 45	734 ± 30	1045 ± 34[b]	845 ± 34	1136 ± 45[b]	747 ± 43
—	—	1289 ± 58[c]	1024 ± 38	1135 ± 75[c]	919 ± 41
—	—	452 ± 14[c]	367 ± 23	566 ± 27[b]	398 ± 10
570 ± 20	577 ± 25	1469 ± 79[b]	912 ± 24	1122 ± 30[b]	912 ± 25
ns		8% > 25%		8% > 25%	
		Mean tryptophan concentrations ± SE (ng/g)			
1636 ± 60[b]	2865 ± 164	3980 ± 290[c]	3040 ± 38	4769 ± 239[b]	3090 ± 70
1424 ± 92[b]	2677 ± 196	3738 ± 296[b]	2790 ± 63	4600 ± 323[b]	2870 ± 109
2071 ± 88[b]	3188 ± 237	4296 ± 350	3677 ± 232	4758 ± 286[b]	3138 ± 118
—	—	4243 ± 395[c]	3153 ± 172	5341 ± 364[b]	3794 ± 202
—	—	4663 ± 357[c]	3546 ± 134	5028 ± 197[b]	3205 ± 211
1943 ± 117[b]	3244 ± 126	4015 ± 241[c]	3224 ± 118	4870 ± 266[c]	3796 ± 142
25% > 8%		8% > 25%		8% > 25%	
16.4 ± 0.4[b]	45.6 ± 0.7	22.0 ± 0.5[b]	69.9 ± 1.0	37.9 ± 1.6[b]	108.6 ± 3.4
1334 ± 25[b]	1520 ± 26	1578 ± 37[b]	1815 ± 21	1660 ± 31[c]	1828 ± 47[c]

total and free tryptophan values (Table 2) and were converted to molar values. These results showed that on days 0, 5, and 11 there was little differences between the molar ratio tryptophan:albumin of the control and malnourished animals. However, on days 16, 21, and 30 the 25% rats showed significantly greater tryptophan:albumin ratios than the 8% rats. To determine if the significant decrease in the tryptophan:albumin ratios seen for the 8% groups at these time points might represent changes in the chemical or physical properties of the plasma tryptophan and/or albumin for these animals, a "theoretical" ratio was calculated. These values were obtained by using the total plasma tryptophan concentrations of the 8% animals (Table 2), and are shown as open bars over the 8% groups for days 16, 21, and 30 (see Fig. 1a). Under these "theoretical" conditions, the only significant differences between the 8% and 25% animals occurred on day 16 with the 25% rats having higher values.

The developmental changes in albumin and NEFA concentrations for the two diet groups are illustrated in Fig. 1b. Both albumin and NEFA

TABLE 2

Developmental Changes in Plasma Tryptophan, Nonesterified Fatty Acids,

Age (days)	0 (Birth)		5		11	
Diets (*N*)	8% 8	25% 8	8% 8	25% 8	8% 8	25% 8
			Mean ± SE (μg/ml)			
Total tryptophan	17.53 ± 0.82[a]	10.32 ± 0.40	3.92 ± 0.30	3.29 ± 0.26	7.24 ± 0.35[a]	10.32 ± 0.22
Free tryptophan	16.98 ± 0.79[a]	9.63 ± 0.36	2.47 ± 0.19[a]	1.73 ± 0.15	4.71 ± 0.18[a]	3.72 ± 0.16
Percentage free	96.87 ± 0.62[a]	93.25 ± 1.08	63.37 ± 2.84[b]	53.37 ± 4.11	65.37 ± 1.24[a]	36.03 ± 1.43
			Mean ± SE (μeq/ml)			
Nonesterified fatty acids	0.49 ± 0.03[a]	0.15 ± 0.02	0.28 ± 0.02[a]	0.14 ± 0.03	0.52 ± 0.04[a]	0.27 ± 0.04
			Mean ± SE (mg/ml)			
Albumin	6.14 ± 0.65	5.83 ± 0.79	8.07 ± 0.79[a]	13.56 ± 1.01	13.31 ± 0.54[a]	33.19 ± 1.53
Total protein	59.19 ± 5.99	56.63 ± 3.63	31.85 ± 1.75	31.44 ± 2.02	59.67 ± 4.41	53.55 ± 1.99
Percentage Albumin	10.97 ± 1.47	10.53 ± 1.18	26.09 ± 3.28[b]	43.73 ± 3.31	22.75 ± 1.13[a]	62.65 ± 3.90

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$; two-tailed t tests.

concentrations from Table 2 were converted to 10^{-4} molar values. At most ages the 25% animals had significantly elevated albumin values, from 170% to 200% of the 8% rat values. Only on days 0 and 16 were there no differences between the groups. The malnourished rats, however, showed significant increases in NEFA concentrations as compared to the control rats, with the 8% values from 125% to 300% of the 25% values at all ages except days 16 and 30 when there were no differences between the two diet groups.

Total plasma protein concentrations from Table 2 showed no marked differences between the normal and malnourished rats until weaning (day 21). At that age and on day 30, the 25% animals showed significant elevations of total plasma protein concentrations to 140% to 170% of the 8% rats. The percentage albumin present in total protein (Table 2), however, showed significant differences between the normal and malnourished rats at several age points. On those days the 25% animals had significant elevations in percentage albumin as compared to the 8% rats with the 25% values being about 120% to 200% of the malnourished group.

To see if there was a correlation between brain tryptophan and plasma tryptophan concentrations for the two diet groups, whole brain tryptophan concentrations for the 8% and 25% rats at each age point (Table 1) were plotted against their respective plasma tryptophan concentrations (Table 2) in Fig. 2. A positive and significant correlation ($r = 0.98$) was seen when brain tryptophan was plotted against free plasma tryptophan (Fig.

Albumin, and Total Protein Concentrations in Normal and Malnourished Rats

16		21		30	
8% 8	25% 8	8% 8	25% 8	8% 8	25% 8
		Mean ± SE (μg/ml)			
2.68 ± 0.13[a]	7.82 ± 0.80	8.13 ± 0.44[a]	16.96 ± 0.37	7.26 ± 0.25[a]	16.93 ± 1.04
1.57 ± 0.09	1.92 ± 0.21	3.70 ± 0.19[a]	2.48 ± 0.20	3.20 ± 0.16[a]	2.06 ± 0.09
58.60 ± 1.37[a]	26.21 ± 4.14	45.71 ± 1.43[a]	14.72 ± 1.38	44.45 ± 2.74[a]	12.45 ± 0.86
		Mean ± SE (μeq/ml)			
0.35 ± 0.03	0.31 ± 0.02	0.59 ± 0.04[b]	0.44 ± 0.03	0.65 ± 0.09	0.53 ± 0.04
		Mean ± SE (mg/ml)			
16.93 ± 1.60	15.94 ± 2.03	25.45 ± 1.85[a]	47.33 ± 1.04	24.32 ± 1.76[a]	42.30 ± 2.29
49.62 ± 2.29	49.36 ± 3.41	36.13 ± 1.61[a]	63.99 ± 3.19	41.09 ± 1.13[a]	59.33 ± 1.26
35.06 ± 4.22	32.07 ± 2.54	69.94 ± 2.53	74.90 ± 3.33	59.06 ± 3.64[c]	71.38 ± 3.78

2a). A positive but not statistically significant correlation was obtained when brain tryptophan was plotted against total plasma tryptophan ($r = 0.53$) in Fig. 2b.

DISCUSSION

Several interesting effects of developmental protein malnitrition emerge from the present studies. First, elevated concentrations of brain serotonin and 5-HIAA were observed in the malnourished animals at most ages examined. As earlier reported by workers in our laboratory (35), the increases in amine and metabolite concentrations were primarily located in the diencephalon and those brain stem regions which contain the serotonin perikarya of the central nervous system. Although the serotonin and 5-HIAA concentrations in the telencephalon of the malnourished animals were less elevated, some increases in this region indicate the terminals of the serotonin-containing neurons are also somewhat affected by developmental protein malnutrition. These increases in brain serotonin and 5-HIAA concentrations can be directly correlated with the corresponding increases in brain tryptophan concentrations observed in the protein-malnourished rats. With the exception of day 16, the regional distribution of brain tryptophan shows that wherever the precursor was elevated in these animals, the amine and metabolite concentrations were also elevated.

A priori, the observed increase in brain tryptophan concentrations in the malnourished rats would not have been expected in an experimental paradigm where the amount of tryptophan present in the 8% casein diet fed to their dams through gestation and lactation was approximately one-third of

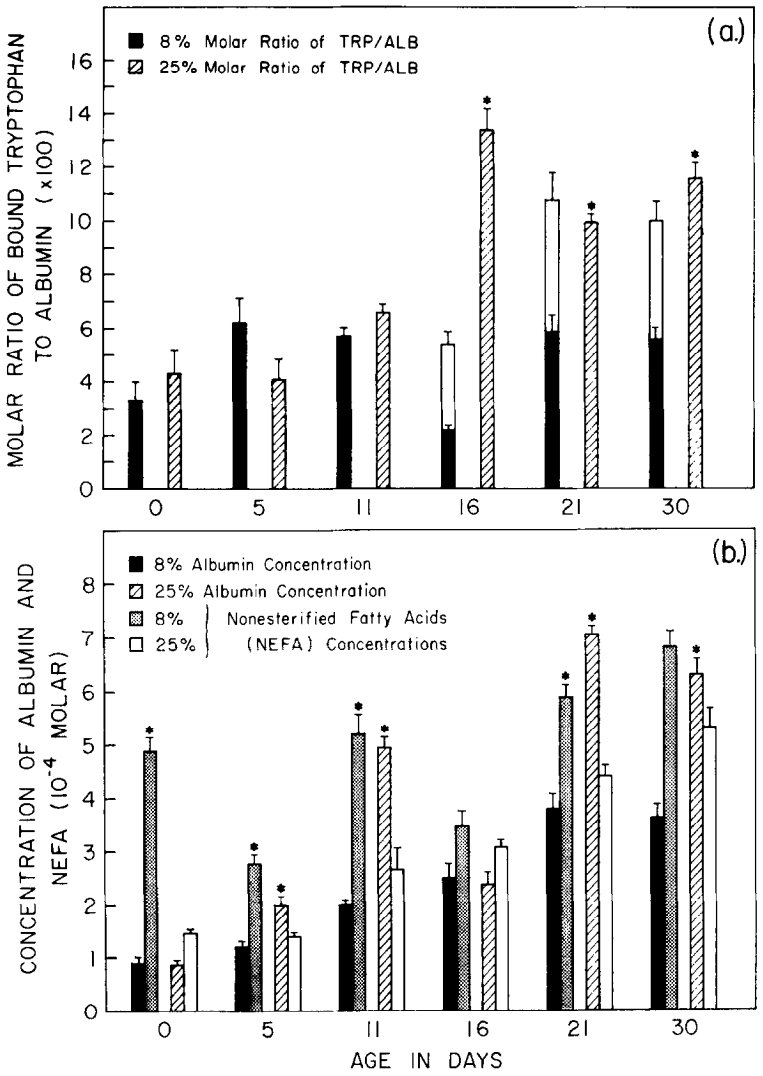

FIG. 1. Ontogenetic development of plasma constituents (mean ± SE) in normal and protein-malnourished rats. (a)—Molar ratio of bound tryptophan to albumin. Solid bars represent the actual tryptophan: albumin (TRP/ALB) ratios seen in the animals. The open bars above the 8% animals represent a "theoretical" TRP/ALB ratio obtained using their total (free and bound) plasma tryptophan concentrations shown for days 16, 21, and 30 as the numerator. Statistical comparisons are for observed TRP/ALB ratios of 8% versus 25% rats. (b)—Albumin and nonesterified fatty acids (NEFA) concentrations in rat plasma. The solid and cross-hatched bars represent the molar albumin concentrations of the animals. The stippled and open bars are the corresponding molar NEFA concentrations. Statistical comparisons are 8% versus 25% for albumin or NEFA levels. Asterisks indicate $P < 0.001$ for 8% versus 25% values, two-tailed t-tests.

the amount present in the 25% casein diet fed to the dams of the control group. Although the malnourished rats tended to eat more grams food per 100 grams body weight, the amounts of their total tryptophan intake per 100 grams body weight was still significantly less than the normal rats (unpublished observations from our laboratory). The decreased intake of tryptophan in malnourished rats is probably reflected in the observation that the total plasma tryptophan concentrations in the malnourished animals were notably lower than the control rats at most ages examined.

A possible explanation of the increased brain tryptophan concentrations of the protein-malnourished rats can be seen upon examination of their free plasma tryptophan concentrations. At most ages these animals showed substantial increases in both the total amount and percentage of free tryptophan in plasma. Recently, several investigators have demonstrated that when free plasma tryptophan concentrations are elevated under conditions of immobilization stress or acute food deprivation (9, 19, 38), by tryptophan loading (5, 38), or through drugs which cause increased brain serotonin turnover (9, 17, 37), corresponding increases in brain tryptophan were observed. Those investigators also reported that whereas brain serotonin concentrations were only slightly elevated by such manipulations (except in tryptophan loading experiments), considerable increases in brain 5-HIAA concentrations occurred. The latter indicates that more serotonin had been manufactured. Those findings, obtained from adult rats in experiments of short duration and usually under nonphysiological conditions, show that only free tryptophan in plasma is available for tryptophan utilization in the brain. Our results confirm and extend those findings considerably. We demonstrate that under a long-term physiological condition (developmental protein malnutrition) the increase of free plasma tryptophan is highly correlated with the increase in brain tryptophan concentrations and that corresponding increases in brain serotonin and 5-HIAA occur. Interestingly, our control animals also show the same relation between free plasma tryptophan and brain tryptophan levels. Although their free plasma tryptophan concentrations are lower than those in malnourished rats, they show correspondingly lower brain tryptophan and for the most part lower brain amine and metabolite concentrations.

Perhaps the reason for the increased amounts of free plasma tryptophan in the malnourished animals lies in the lower molar binding of tryptophan to albumin seen in these animals. It was reported that little binding between tryptophan and albumin occurs in the newborn rat (5) and we find this to be the case for both diet groups at birth. Subsequent developmental changes in this ratio show that by the 16th postnatal day there are considerable differences between the tryptophan-to-albumin binding of the control and malnourished rats. The control group shows a normal molar binding ratio of about 0.1 (21), whereas the malnourished group has binding ratios on

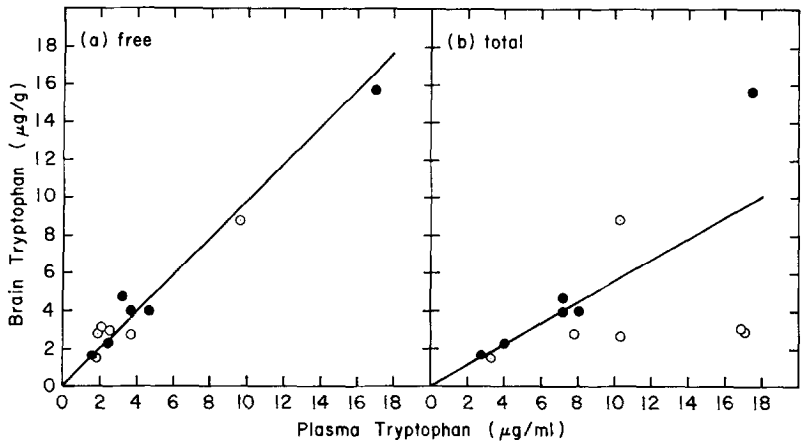

FIG. 2. Relationships between brain and plasma tryptophan concentrations. Each point represents the whole brain versus the plasma concentration of tryptophan for a group at the different ages shown in Tables 1 and 2. (a)—Brain tryptophan versus free plasma tryptophan. ○, Normal rats; ●, malnourished rats. r for both groups = 0.98 ($N = 12$; $P < 0.001$). (b)—Brain tryptophan versus total plasma tryptophan. ○, Normal rats; ●, malnourished rats. r for both groups = 0.53 ($N = 12$; n.s.). Regression lines are drawn by visual approximation to give best fit.

the order of 0.02 to 0.06. This lower ratio is not due to the differences between the total plasma tryptophan concentrations observed in the control and malnourished animals. Calculations using the total plasma tryptophan values for the malnourished animals show that they have the same "theoretical" molar ratio of tryptophan: albumin as the control rats (except on day 16). The fact that the malnourished rats had considerably lower binding of tryptophan to albumin suggests that some alteration in normal regulation of tryptophan binding occurred, causing more tryptophan to be present in the free form and available for brain tryptophan metabolism.

It is known that tryptophan and nonesterified fatty acids compete for the same binding sites on albumin (18, 21). Recently, it was reported (7) that the addition *in vitro* of NEFA to rat plasma within the range of physiologic concentrations results in an increased concentration of free tryptophan. Also, several reports (8–10) have indicated that many drugs and hormones known to increase lipolysis and NEFA concentrations by changing cyclic adenosine 5′-monophosphate metabolism in fat cells (24) can decrease the binding of plasma tryptophan causing more to be present in the free form. These findings may serve as part of an explanation for the decreased binding of tryptophan to albumin seen in the malnourished rats. The malnourished rats show NEFA concentrations that are considerably greater than the control animals at most ages examined. An additional reason for

decreased tryptophan binding in the plasma of the malnourished rats may be the decreased concentrations of albumin seen at the same ages in the malnourished rats. Because the molar ratio between NEFA and albumin is aproximately 1.0 (18, 32), and can be increased to as high as 2.0 during NEFA loading experiments (31), the increase in NEFA concentrations and decrease in albumin concentrations in the malnourished rats are working together to keep the tryptophan from successful competition for binding sites on the albumin molecule. This combination of events does not occur in the control animals. Even when their NEFA concentrations are high, the controls have larger concentrations of albumin, hence providing adequate albumin binding sites for tryptophan.

It could be argued that the decreased albumin concentrations of the malnourished rats are the consequence of lower total plasma protein levels. This does appear to be the case. Until weaning, both groups of animals had essentially the same amounts of total protein in plasma. However, the fraction of albumin present in total protein was considerably lower for the malnourished rats at most ages, indicating that some developmental alteration in albumin synthesis had occurred. Thus, the decreases in albumin concentrations coupled with the increased concentrations of NEFA appear to be causing more free tryptophan to be present in the plasma of the malnourished rats. This presumably results in subsequent elevations of their brain tryptophan levels and in higher serotonin and 5-HIAA concentrations.

Significant increases in the brain concentration of serotonin and 5-HIAA were observed for the malnourished rats at all ages examined in the present study except on days 5 and 16. On those days there were few or no differences in brain amine and metabolite concentrations between the two diet groups. Also, at those two ages, both the control and malnourished rats showed significant decreases in their total and free plasma tryptophan concentrations as compared to either the preceding or subsequent age points, i.e., days 0 and 11 are greater than day 5; days 11 and 21 are greater than day 16. This implies some ontogenetic alterations in systemic tryptophan metabolism which are not due to any dietary manipulations. It has been reported that two age-dependent peaks in cerebral amino acid concentrations occur at about ages 5 and 18 days for rat (23). This change in the influx of brain amino acids may, in part, account for the decreases in brain and plasma tryptophan concentrations and subsequent decreases in brain serotonin and metabolite seen for both groups of animals. One other possible factor for day 16 is the initiation of tryptophan pyrrolase activity in liver. This enzyme becomes active at about day 15 in the rat and attains adult values within 24 h (4). Perhaps the initiation of the major physiological

pathway for tryptophan metabolism may influence the systemic concentrations of plasma tryptophan causing their temporary decrease.

It is also of interest to mention that significant increases in plasma and brain tryptophan occur before the start of the brain-growth spurt period. Previously, the effects on the developing central nervous system were attributated primarily to that period (13). We find, however, that on day of birth the malnourished rats show significant elevations in both peripheral and brain tryptophan concentrations along with increased concentrations of brain serotonin and metabolite as compared to the control animals. Although it has been reported that peripheral and brain tryptophan concentrations are elevated in newborn rat and persist for several hours after birth (5), the increase in the amount of brain serotonin and metabolite levels in the malnourished rats in this investigation are of much greater magnitude.

The increased amounts of brain tryptophan, serotonin, and 5-HIAA seen in the malnourished animals at most ages as a consequence of the developmental protein malnutrition treatment can be directly related to the increased amounts of free plasma tryptophan available for their brain metabolism. This finding indicates that the malnourished rats may be shunting more tryptophan to the brain at the expense of the periphery, which may be related to the large differences in body weights between the two groups of animals, i.e., the malnourished rats had body weights 30% to 50% of the control groups. Therefore, from a teleological standpoint, the elevations in brain serotonin and metabolite seen in the malnourished rats may be only the consequence of a need to assure that more tryptophan is available to ensure adequate brain protein synthesis and development in early life. This may, in part, account for the relatively small differences in the brain weights of the malnourished animals which were 85% to 95% of the control group.

The consequences of elevations in brain serotonin in the adult animal due to developmental changes in tryptophan availability remain to be determined. We have reported alterations in response to stress (34), seizure susceptibility (33), and sleep paterns (16) in adult animals that were protein malnourished during development. Those changes may be related to a disturbance in brain serotonin functions in the adult malnourished rat. Certainly more work is indicated to explain more fully the consequence of alterations in tryptophan metabolism on the subsequent functioning of the central nervous system.

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