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Tryptophan Concentrations in Rat Brain

FAILURE TO CORRELATE WITH FREE SERUM TRYPTOPHAN OR ITS RATIO TO THE SUM OF OTHER SERUM NEUTRAL AMINO ACIDS

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Groups of rats were deprived of food overnight and then given free access to diets designed to raise (carbohydrate) or lower (carbohydrate and large neutral amino acids) brain tryptophan concentrations. Similar diets were supplemented with 40% fat and fed to other groups. All animals were killed 2h after food presentation. Sera from animals fed carbohydrate plus fat contained 2.5 times as much free tryptophan as sera from rats given only carbohydrate; however, brain tryptophan concentrations did not differ. Similarly, sera from rats fed on carbohydrate, large neutral amino acids, and 40% fat contained 5 times as much free tryptophan as those from rats given this meal without fat, but brain tryptophan concentrations increased by only 26%. Correlations were made between brain tryptophan and (1) free serum tryptophan, (2) the ratio of free serum tryptophan to the sum of the other large neutral amino acids in serum that compete with it for uptake into the brain, (3) total serum tryptophan or (4) the ratio of total serum tryptophan to the sum of its circulating competitors. The *r* values for correlations (3) and (4) (i.e. those involving total serum tryptophan) were appreciably higher than those for correlations (1) and (2). Brain tyrosine concentrations also were found to correlate well with the ratio of serum tyrosine to the sum of its competitors. Competition for uptake into the brain among large neutral amino acids (represented here by serum ratios) thus appears to determine the changes in the brain concentrations of these amino acids under physiological conditions (i.e. after food consumption). Total, not free, serum tryptophan is the relevant index for predicting brain tryptophan concentrations.

The concentration of tryptophan in the brain appears to indicate a variation in response to changes in the serum concentrations of total tryptophan (Fernstrom & Wurtman, 1971*a*,*b*), free tryptophan (Tagliamonte *et al.*, 1971; Knott & Curzon, 1972) and other large neutral amino acids (Fernstrom & Wurtman, 1972) that compete with tryptophan for uptake into the brain (Blasberg & Lajtha, 1965). Almost all of the studies designed to show that the serum concentration of free tryptophan controls brain tryptophan concentration, however, have involved relatively unphysiological experimental conditions, such as starving rats for long periods (Knott & Curzon, 1972; Tagliamonte *et al.*, 1973), subjecting them to immobilization stress (Knott & Curzon, 1972), or injecting them with drugs that dissociate tryptophan from its carrier molecule in blood, serum albumin (Guerinot *et al.*, 1974; Curzon & Knott, 1974).

In contrast, the dependence of brain tryptophan on the serum concentrations of total tryptophan and the other large neutral amino acids has been demonstrated in minimally traumatized animals that modify their brain tryptophan concentration by consuming food (e.g. Fernstrom & Wurtman, 1972; Fernstrom *et al.*, 1973). In some of these studies, it has also been possible to dissociate diet-induced changes in free serum tryptophan concentrations from those in brain tryptophan (Madras *et al.*, 1973, 1974; Fernstrom *et al.*, 1975*b*).

Biggio *et al.* (1974) and Perez-Cruet *et al.* (1974) suggested that the concentrations in serum of both free tryptophan and the other large neutral amino acids may be important in determining brain tryptophan concentrations. On that basis, it would seem reasonable to expect that the ratio of free serum tryptophan to the sum of its competitors would be an excellent predictor of tryptophan concentrations in the brain. We undertook the present studies in part to test this hypothesis, and to determine whether the brain concentrations of tyrosine, another large neutral amino acid, also can be predicted by the ratio of its serum concentration to the sum of the other large neutral amino acids. The results demonstrate that the ratio involving free tryptophan is not an accurate predictor of brain tryptophan concentration; the ratio involving total tryptophan is a much better indicator. For total serum and brain tryptophan

concentrations, brain tyrosine concentrations best reflect alterations in the serum ratio of tyrosine to the sum of its competitors.

Materials and Methods

Groups of ten male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.), each weighing 150–200g, were acclimatized to our animal quarters for at least 1 week before experimentation. During this time, they had free access to food (Charles River Rat, Mouse, and Hamster Maintainance Diet, containing 24% protein) and water and were exposed to light (300μW/cm^2; Vita-Lite, Duro-Test Corp., North Bergen, NJ, U.S.A.) between 08:00 and 20:00h daily. At 19:00h the day before an experiment, the rats were deprived of food; at 09:00h the next morning, groups of ten of these animals were given free access to one of the experimental diets (Table 1). Control animals continued to be deprived of food, but had free access to water. In our animal quarters, rats ingest 30–35% of their daily food intake during the daylight hours, an observation also made by others (e.g. see Zucker, 1971). Consequently, the animals used in these experiments were not starving.

The animals were decapitated 2h after the food was presented, and the amount of food consumed was measured. Rats that ingested carbohydrate alone consumed about 10g each, whereas those that consumed carbohydrate plus 40% fat ate about 7.5g (Table 1). Trunk blood was collected and centrifuged; the serum was separated and frozen until assayed. Tubes were stored at −20°C in an atmosphere of N_2+CO_2 (95:5) in order to maintain the physiological pH. Brains were removed quickly, frozen on solid CO_2, and stored at −70°C until assayed for tryptophan and tyrosine content.

Before analysis, sera from two animals in each diet group were pooled, thus decreasing the total number of samples assayed to 25. This procedure allowed all of the serum constituents described below to be measured in a single experiment. It was not necessary to pool brain samples in order to assay their constituents; hence all 50 were assayed individually for tryptophan and tyrosine. These data were then pooled in groups of two, which corresponded to the two donor animals whose sera had been pooled. Data obtained from this experiment were very similar to those from other studies, in which individual, unpooled sera were assayed for one or two compounds.

Sera were subjected to equilibrium dialysis at 37°C and pH7.5 for 3.5h; free serum tryptophan concentrations were determined from samples of the diffusate (Lipsett *et al.*, 1973; Madras *et al.*, 1974). No significant increase in serum non-esterified fatty acid concentration occurred during the dialysis period, a finding that confirms the observation of Madras *et al.* (1974). Free serum, total serum and brain tryptophan were assayed fluorimetrically by the method of Denckla & Dewey (1967), as modified by Lehmann (1971) and Bloxam & Warren (1974). The concentration of tyrosine in brain was measured by the fluorimetric method of Waalkes & Udenfriend (1957). A Beckman model 121 automatic amino acid analyser was used to measure tyrosine, phenylalanine, and the branched-chain amino acids in serum.

When amino acids were included in the experimental diet, an equivalent amount of carbohydrate was removed to keep the diets isocaloric. Each amino acid was added in an amount necessary to attain the concentration normally found in an 18% casein diet (Fernstrom & Wurtman, 1972). When fat was included in the diet (400g), an equivalent amount of carbohydrate was removed (400g). The calorific value (kJ/g) of fat is more than twice that of carbohydrate; the potential dichotomy in energy consumption did not materialize, however, because the rats ingesting the 40% fat diets consumed less food than those ingesting the fat-free diets. Hence the actual calorific intake of both groups was similar: carbohydrate without fat (10g each), 77kJ (18.3kcal);

Table 1. *Composition of experimental diets*

In the text the diets are referred to as follows: diet 1, carbohydrate without fat; diet 2, carbohydrate+40% fat; diet 3, neutral amino acid diet without fat; diet 4, neutral amino acid diet+40% fat. Ingredients are expressed as g/kg dry weight. The final diet contains water (1000ml) and yields 2kg. The salt mixture is as described by Rogers & Harper (1965). The amino acids were from Nutritional Biochemicals Corp. (ICN Pharmaceuticals, Cleveland, OH, U.S.A.). Each amino acid is present at the concentration that would normally be found in an 18% casein diet (Fernstrom & Wurtman, 1972). 'Food consumed' represents the amount of food (wet weight) ingested during the 2h experimental period.

	Composition (g/kg)			
Diet ...	1	2	3	4
Ingredient				
Sucrose	259	147	244	132
Dextrose	333	189	313	169
Dextrin	333	189	313	169
Mazola oil	—	400	—	400
Salt mixture	40	40	40	40
Agar	35	35	35	35
Water	1000	1000	1000	1000
Amino acids				
Tyrosine	—	—	10.26	10.26
Phenylalanine	—	—	8.16	8.16
Leucine	—	—	15.00	15.00
Isoleucine	—	—	9.92	9.92
Valine	—	—	11.82	11.82
Food consumed (g)	10	7.5	9.5	8.0

carbohydrate+40% fat (7.5g each), 92kJ (22kcal); neutral amino acid diet without fat (9.5g each), 73kJ (17.4kcal); and neutral amino acid diet+40% fat (8.0g each), 94kJ (22.4kcal).

Student's *t*-test was used to analyse data for significance; sample correlation coefficients were tested against the null hypothesis that $\rho = 0$.

This experiment was performed on five occasions, with essentially the same results.

Results

Effect of 40% dietary fat on meal-related changes in serum amino acid concentrations

Total serum tryptophan concentrations were unchanged 2h after rats consumed the fat-free carbohydrate diet. [In many experiments total serum tryptophan concentration rises in response to carbohydrate ingestion (Fernstrom & Wurtman, 1971*b*)]. Free serum tryptophan concentrations, however, fell significantly below values for rats deprived of food overnight (Table 2); the shift in the ratio of bound to free tryptophan probably reflects an insulin-mediated fall in serum non-esterified fatty acid concentrations (Madras *et al.*, 1973). The inclusion of 40% fat in this diet elicited substantially different alterations in free serum, but not total serum, tryptophan; whereas free serum tryptophan concentrations fell to 13.86nmol/ml in animals consuming carbohydrate alone, it increased to 32.71nmol/ml in rats ingesting carbohydrate plus 40% fat (probably because of the corresponding rise in non-esterified fatty acid concentration). Each of these diets caused decreases (probably mediated by insulin) in serum tyrosine concentrations (Table 3), as well as in the serum concentrations of phenylalanine and the branched-chain amino acids leucine, isoleucine and valine (Table 4). Consequently, the ratio of the total serum tryptophan concentration to the sum of the concentrations of tyrosine, phenylalanine, leucine, isoleucine and valine rose after the ingestion of either diet (Table 2). Similarly, the ratio of serum tyrosine to the sum of tryptophan, phenylalanine, leucine, isoleucine and valine concentrations also increased after the ingestion of either of the carbohydrate diets, even though serum tyrosine concentrations were decreased by both (Table 3).

Table 2. *Effects of food ingestion on free and total serum tryptophan concentrations, the serum concentration ratio of total tryptophan to the sum of its competitors, and brain tryptophan*

Groups of ten male Sprague–Dawley rats were deprived of food overnight and the next morning given free access to one of the four diets. They were killed 2h after food presentation. Data are presented as the mean±S.E.M. 'Tryptophan ratio' is the ratio total serum tryptophan/(tyrosine + phenylalanine + leucine + isoleucine + valine). *$P < 0.01$; **$P < 0.5$ (compared with unfed values).

	Serum tryptophan				
Diet group	Free (nmol/ml)	Free (%)	Total (nmol/ml)	Tryptophan ratio	Brain tryptophan (nmol/g)
Unfed	22.3±1.4	20	102 ±8	0.120±0.011	23.8±1.2
Carbohydrate without fat	13.9±0.8*	13	108 ±6	0.366±0.014*	30.2±0.6*
Carbohydrate+40% fat	32.7±1.4*	32	99.9±5.9	0.257±0.018*	27.0±1.5
Neutral amino acid diet without fat	6.4±0.4*	11	59.7±1.8*	0.082±0.002*	13.1±0.6*
Neutral amino acid diet+40% fat	30.2±0.5*	39	77.7±1.7**	0.094±0.002**	16.5±0.6**

Table 3. *Effects of food ingestion on serum tyrosine concentrations, the serum concentration ratio of tyrosine to the sum of its competitors, and brain tyrosine*

Groups of male Sprague–Dawley rats were deprived of food overnight, and the next morning given free access to one of the four diets. They were killed 2h after food presentation. Data are presented as the mean±S.E.M. 'Tyrosine ratio' is the ratio total serum tyrosine/(tryptophan+phenylalanine+leucine+isoleucine+valine). *$P<0.01$; **$P<0.025$ (compared with unfed values).

	Serum		
Diet	Tyrosine (nmol/ml)	Tyrosine ratio	Brain tyrosine (nmol/g)
Unfed	107 ±3	0.128±0.008	155± 2
Carbohydrate without fat	61.6±4.1*	0.181±0.010*	184± 7
Carbohydrate+40% fat	67.2±2.9*	0.166±0.005*	187± 5*
Neutral amino acid diet without fat	201 ±11*	0.345±0.021*	205± 8*
Neutral amino acid diet+40% fat	175 ±8*	0.242±0.017*	191±11**

Table 4. *Effects of food ingestion on the serum concentrations of phenylalanine, leucine, isoleucine and valine*

This is the same experiment as described in Tables 2 and 3. Data are presented as the mean±S.E.M. The concentration of each amino acid in the sera of animals that consumed one of the four diets is significantly different from the concentrations in the animals deprived of food ($P<0.01$). The only exceptions are the concentrations of isoleucine and valine in rats that consumed the neutral amino acid diet+40% fat, which are not significantly different from unfed values.

Diet	Amino acid concentration (nmol/ml)			
	Phe	Leu	Ile	Val
Unfed	84±3	241±13	141±6	279±11
Carbohydrate without fat	55±3	60± 4	41±2	76± 2
Carbohydrate+40% fat	63±1	91± 4	53±4	115± 7
Neutral amino acid diet without fat	101±1	139± 7	90±3	196± 6
Neutral amino acid diet+40% fat	100±2	178± 8	117±5	258±14

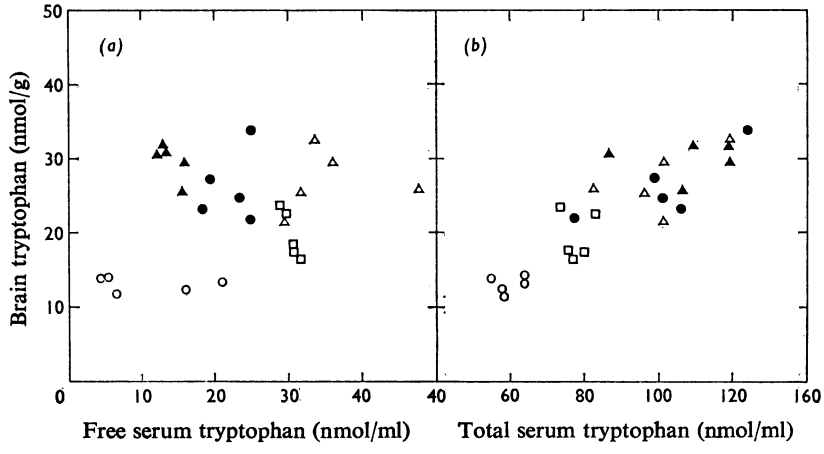

Fig. 1. *Correlations between brain tryptophan concentrations and free (a) or total (b) serum tryptophan in individual rats consuming single meals*

Groups of rats deprived of food were given free access to one of the following diets and killed 2h later: ▲, carbohydrates without fat; △, carbohydrates+40% fat; ○, carbohydrates+large neutral amino acids without fat; □, carbohydrates+large neutral amino acids + 40% fat; ●, unfed controls. For (*a*) $r=0.23$ and for (*b*) $r=0.87$.

The ingestion of the diets containing neutral amino acids produced somewhat different effects: free serum tryptophan concentrations fell when the diet lacked fat (Table 2), as in animals that consumed carbohydrate alone, and rose when fat was added; however, total serum tryptophan decreased after the ingestion of either diet (Table 2). Serum tyrosine (Table 3) and phenylalanine (Table 4) concentrations rose. The serum concentrations of each of the branched-chain amino acids fell, but not to the very low concentrations observed when the meal did not contain these compounds (Table 4). The net effect of these changes was to decrease the ratio of total serum tryptophan to the sum of the other large neutral amino acids (Table 2) and to increase the ratio of serum tyrosine to the sum of its competitors (Table 3).

Effect of 40% dietary fat on meal-related changes in brain tryptophan and tyrosine concentrations

The ingestion of either carbohydrate diet tended to raise tryptophan concentrations in the brain (Table 2). The greater increase (and the only one that was significant in this particular study) was observed in the animals that consumed the fat-free meal; i.e. the rats in which free serum tryptophan had fallen markedly. After the ingestion of a carbohydrate plus neutral amino acid diet, with or without fat, brain tryptophan concentrations decreased significantly ($P<0.01$), even though free serum tryptophan had increased substantially in animals eating 40% fat. Brain tyrosine concentrations rose markedly after each of the dietary treatments.

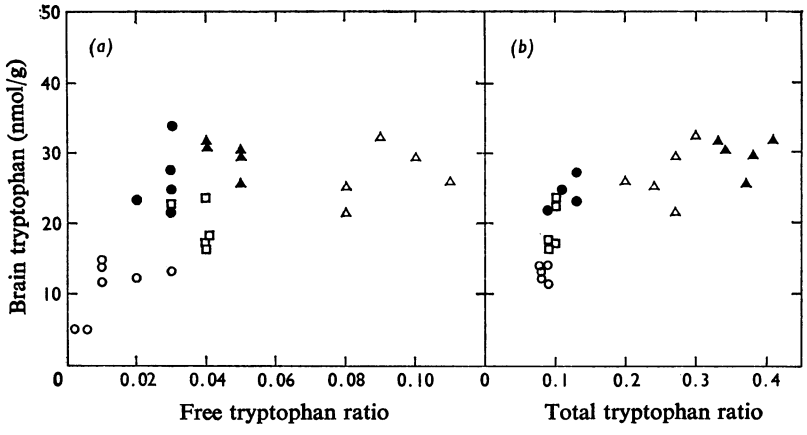

Fig. 2. *Correlations between brain tryptophan and the ratios of both free (a) and total (b) serum tryptophan to the sum of the other neutral amino acids that compete with it for uptake in individual rats consuming single meals*

Data are from the same experiment as described in Fig. 1. The tryptophan ratios are serum (total or free) tryptophan/(tryptophan+phenylalanine+leucine+isoleucine+valine). For (*a*) $r = 0.50$ and for (*b*) $r = 0.79$.

Correlations between serum tryptophan parameters and brain tryptophan concentration

The serum concentrations of free and total tryptophan in individual unfed or fed animals are plotted in Fig. 1 against their brain tryptophan concentrations. Brain tryptophan concentration correlates poorly with free serum tryptophan ($r = 0.23$; not significantly different from $r = 0$), whereas it correlates well with total serum tryptophan ($r = 0.87$; $P < 0.01$ that $r = 0$). When brain tryptophan is plotted against the ratio of serum free tryptophan to the sum of the other large neutral amino acids, the correlation is improved somewhat ($r = 0.50$; $P < 0.05$ that $r = 0$) over that against serum free tryptophan alone, but is not as good as the correlation between brain tryptophan concentration and the ratio of total tryptophan to the sum of its competitors (Fig. 2) ($r = 0.79$; $P < 0.01$ that $r = 0$).

Discussion

These results demonstrate the following. (1) In untreated animals that modify their serum amino acid concentrations by food consumption *ad libitum*, free serum tryptophan concentrations are poor predictors of brain tryptophan concentrations (Fig. 1); this finding confirms earlier observations by Madras *et al.* (1973, 1974) and Fernstrom *et al.* (1975*b*). (2) The ratio of free serum tryptophan to the sum of the other large neutral amino acids, although providing a better correlation with brain tryptophan concentration than free serum tryptophan alone, does not improve dramatically the utility of free tryptophan as a predictor of brain tryptophan. (3) The best parameters in serum for predicting brain tryptophan concentrations are total serum tryptophan and the ratio of total tryptophan to the sum of the other large neutral amino acids (Figs. 1 and 2). (4) The utility of such ratios for predicting the concentrations of neutral amino acids in the brain is confirmed by the observation that brain tyrosine concentration correlates better with the serum tyrosine to competitor ratio than with tyrosine concentrations alone (Table 3).

These studies illustrate the extent to which changes in free serum tryptophan can be dissociated from those in brain tryptophan. (1) The addition of 40% fat to the carbohydrate diet produced a 2.5-fold difference in free serum tryptophan (13.86 against 32.71 nmol/ml; see Table 2) but decreased brain tryptophan concentrations slightly (from 30.22 to 27.03 nmol/g). (2) Adding fat to the neutral amino acid diets caused a 5-fold difference in serum free tryptophan concentrations (6.42 against 30.24 nmol/ml), but only a slight difference in brain tryptophan concentrations (13.08 against 16.50 nmol/g). (3) If the concentration of free tryptophan in serum were important in controlling brain tryptophan, the brain tryptophan would not have fallen after the ingestion of the neutral amino acid diet plus 40% fat (from 23.75 to 16.50 nmol/g), because free serum tryptophan concentrations actually increased (from 22.28 to 30.24 nmol/ml). (4) Similarly the fall in free serum tryptophan in response to the ingestion of carbohydrate without fat (from 22.28 to 13.86 nmol/ml) should have led to a decrease in brain tryptophan concentrations, but did not. Instead, brain tryptophan concentrations actually increased (from 23.75 to 30.22 nmol/g; Table 2) after ingestion of this diet.

Biggio *et al.* (1974) and Perez-Cruet *et al.* (1974) have suggested that both competition with other

neutral amino acids and binding to serum albumin might be important in determining the relationship between serum amino acid concentrations and brain tryptophan concentrations. If this notion were correct, then the ratio of free serum tryptophan to the sum of the other large neutral amino acids would be expected to provide the best index of brain tryptophan concentration. Our results contradict this expectation. The correlation between brain tryptophan and the ratio of free serum tryptophan to the sum of its competitors for uptake is not nearly so good as that obtained when total serum tryptophan is used (Fig. 2).

In these experiments, total serum tryptophan appeared to predict brain tryptophan as well as, if not better than, the serum ratio ($r = 0.87$ against $r = 0.79$ respectively). The choice of the experimental diets readily explains this result: previous studies (Fernstrom *et al.*, 1975*a*) have shown that the ingestion of diets containing neutral amino acids decreases both total serum and brain tryptophan concentrations, and that the consumption of carbohydrate diets increases (although occasionally insignificantly) both of the tryptophan pools (Fernstrom & Wurtman, 1971*b*). Had we included casein diets (i.e. foods containing all of the amino acids in physiological proportions) in these studies, total serum tryptophan would have been readily dissociated from brain tryptophan (Fernstrom *et al.*, 1973). Thus although brain tryptophan concentrations accurately reflects total serum tryptophan in certain instances, this relationship does not hold in all cases. In contrast, the relationship between brain tryptophan concentration and the ratio of serum total tryptophan to the sum of its serum competitors for uptake seems to be generally valid (Fernstrom *et al.*, 1975*a*,*b*).

Brain tyrosine concentrations also change in parallel with the serum ratio of tyrosine to the sum of its competitors (Table 3), but not always with serum tyrosine concentrations alone. Carbohydrate ingestion, for example, decreased serum tyrosine concentrations, but increased both the serum ratio and brain tyrosine concentrations. This increase in the serum ratio reflects the fact that insulin decreases the serum concentrations of the branched-chain amino acids to a much greater extent than those of tyrosine (Table 4). [This also applies with phenylalanine; brain phenylalanine concentrations and the serum ratio with phenylalanine in the numerator also rise after carbohydrate ingestion by rats (Fernstrom & Faller, 1975).] That tyrosine uptake into brain is also competitive with the other large neutral amino acids has been extensively documented in pharmacological studies (e.g. Guroff & Udenfriend, 1962; Blasberg & Lajtha, 1965). The present study suggests that this competition is also an important factor under more physiological conditions, i.e. those in which the sole treatment is the elective consumption of food and in which serum neutral amino acid concentrations vary within a range observed in normal animals. In a preliminary report, we noted that diet-induced changes in the brain concentrations of most of the large neutral amino acids appear best to reflect the coincident alterations in the ratio of their serum concentration to the sum of the five competitors (Fernstrom & Faller, 1975).

The fact that, after treatments lasting several hours, brain tryptophan concentrations correspond better with total serum tryptophan (corrected for competing neutral amino acid concentrations) than with free serum tryptophan does not, of course, imply that the circulating tryptophan–albumin complex actually enters the brain tissue, any more than the marked decrease in serum non-esterified fatty acid concentration (almost all of which is bound to albumin) after insulin administration means that albumin is entering adipocytes and other peripheral cells. A more likely formulation is that the affinity of intact brain for tryptophan is greater than that of serum albumin; hence, the brain is able to dissociate the albumin–tryptophan complex as its passes through central-nervous-system capillaries.

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