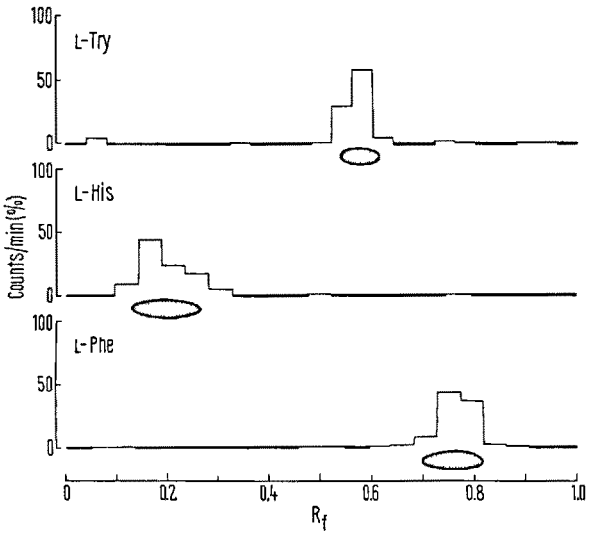
Amino Acid Accumulation by Brain Slices: Interactions Among Tryptophan, Phenylalanine and Histidine

In recent years, numerous papers have dealt with the characteristics of amino acid uptake by brain tissue. Uptake of tryptophan has special interest since it has been reported that the intracellular concentration of this amino acid might regulate polyribosome aggregation, and in this way exerts a major modulating effect on the rate of protein synthesis[1,2]. However, there are few studies on the effect of other amino acids on the cerebral accumulation of tryptophan. High circulating levels of phenylalanine cause depletion of tryptophan in rat brains in vivo[3], and the same effect has recently been found in two in-vitro systems, brain synaptosomal fractions[4], and brain slices[5]. Our report confirms the inhibitory effect of phenylalanine upon the accumulation of tryptophan by brain slices, whereas in presence of histidine we found an increase in the steady state concentration of tryptophan in the tissue.

Material and methods. Cortical brain slices (0.3 mm thick, about 70 mg weight) were prepared from adult male albino rats, as described by McILLWAIN[6]. Each slice was incubated at 37 °C, with agitation, in 5 ml of gassed (5% CO_2–95% O_2) Krebs-Ringer bicarbonate saline, pH 7.4[7], which contained glucose (10 m*M*) and the amino acids under investigation, with tracer amounts of the appropriate radioactive isomer. After incubation, the slices were quickly drained on glass, weighed with a torsion balance, and homogenized in 2 ml of 6% cold trichloroacetic acid (TCA). Samples of 0.1 ml of the incubation medium, before and after incubation, were also mixed with 2 ml of TCA. Aliquots of 0.2 ml, from the centrifugated clear supernatants, were added to 10 ml phosphor[8] and counted for radioactivity. Counting error due to quench variation was insignificant, and no correction was applied to individual sample counts. Tissue water content at the end of incubation was determined by drying slices at 105 °C for 10 h, and the amino acid concentration was calculated as µmole/ml tissue water. Unlabelled amino acids were obtained from Sigma; L-phenylalanine-C^{14}(U), L-histidine (ring-2-C^{14}) and L-tryptophan (methylene-C^{14}), from the Radiochemical Centre, Amersham.

Since the concentration of the amino acids was calculated from the radioactivity in the slices, identification of labelled material in the tissue was carried out by paper chromatography[9]. TCA extracts of slices incubated with histidine-C^{14} or phenyl-alanine-C^{14}, and boiling water extracts of slices incubated with tryptophan-C^{14}, were subjected to chromatography on Whatman No 3 paper strips in 2 solvents, butanol: acetic acid: water (12:3:5) and water-saturated phenol. After 10 h runs, the strips were dried, cut into 1 cm lengths, and each piece placed in 10 ml phosphor for counting of radioactivity. Duplicated strips were run with unlabelled markers and developed with ninhydrin. For the 3 amino acids studied, it was found that 90–98% of the total radio-activity in the strip was located at the same Rf value of the respective amino acid (Figure).



Distribution of radioactivity in chromatograms of brain extracts after 60 min incubation with labelled tryptophan, histidine or phenylalanine, run in butanol:acetic acid:water. Hatched areas represent the positions of the ninhydrin stained spots in parallel chromatograms of unlabelled amins acids.

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Table I. Accumulation of L-tryptophan, L-penylalanine and L-histidine by brain slices

Amino acid	Final tissu/medium concentration ratio				
	When present alone	In the presence of an equimolar amount of L-phe	L-his	L-try	When the 3 amino acids are Present
L-try	4.52 ± 0.11 (46)	1.85 ± 0.07 (19)	6.78 ± 0.34 (20)	—	3.43 ± 0.22 (10)
L-phe	1.84 ± 0.02 (38)	—	3.76 ± 0.18 (10)	1.63 ± 0.03 (18)	2.84 ± 0.17 (10)
L-his	14.85 ± 0.80 (27)	7.29 ± 0.79 (10)	—	6.88 ± 1.77 (10)	9.52 ± 0.46 (10)

Brain slices were incubated for 60 min with the appropriate amino acid(s), and the final tissue/medium concentration ratios were calculated as µmole per ml of tissue water/µmole per ml of medium. The initial concentration of each amino acid in the medium was 1 m*M*. Figures are means ± S.E.M. In brackets the number of slices.

Table II. Effect of histidine in the medium or preincubation with histidine (his) on the initial uptake of tryptophan by brain slices

	No of slices	Tryptophan uptake (μmole/ml of tissue water)
Without preincubation		
Controls	29	1.39 ± 0.06
In presence of his 1 m*M*	18	1.29 ± 0.05
5 m*M*	10	0.91 ± 0.05
With preincubation		
Controls	12	1.04 ± 0.06
Loaded with his	14	1.35 ± 0.06

Tryptophan uptake was determined after 3 min incubation with tryptophan 1 m*M* in the medium. In the experiments with princubation, brain slices were preincubated for 30 min without amino acids (controls) or in presence of histidine 5 m*M* (loaded); after a three-second washing in cold medium, they were incubated with tryptophan. The concentration of histidine in the slices at the end of preincubation with this amino acid was 22.9 ± 0.8 μmole/ml tissue water. Figures are means ± S.E.M.

Results and discussion. When brain slices are incubated in the presence of an amino acid for 60 min, the accumulation of the latter in the tissue approaches a steady level, which is dependent on its initial concentration on the suspending medium and on the presence of other amino acids. The data of Table I were obtained with these types of experiments. It is shown that the accumulation of tryptophan or histidine is decreased in presence of equimolar amounts of phenylalanine. Similarly, the accumulation of phenylalanine or histidine is decreased in presence of tryptophan. However, the accumulation of tryptophan or phenylalanine is increased in the presence of equimolar amounts of histidine.

The inhibiton of the accumulation of histidine by phenylalanine and by tryptophan is in agreement with the results of NEAME[10].

Accumulation experiments, such as those shown in Table I, while simulating more closely the physiological steady state condition, cannot explain in detail the dynamics of the uni-directional fluxes occurring at the cell membrane. Therefore, we have further studied, in short term incubations, the effect of histidine on the uptake of tryptophan. Table II shows that the initial (3 min) uptake of tryptophan by fresh tissue is decreased by the presence of histidine in the medium, in particular when the concentration of histidine is greater than that of tryptophan. On the other hand, Table II shows, also, that preloading of the slices with histidine enhances the initial uptake of tryptophan; in parallel experiments it was found that the incubation of the histidine preloaded slices with tryptophan (1 m*M*), roughly doubles the initial exit rate of histidine from the tissue. Therefore, from the data of Table II, it seems that both amino acids compete for the membrane carrier when they are on the same side of the cell membrane, but, when they are on opposite sides, intracellular histidine exchanges with extracellular tryptophan. Such a type of exchange diffusion could explain the enhancing effect of histidine on the accumulation of tryptophan in the long term experiments of Table I.

Resumen. La concentración de triptófano en cortes de cerebro incubados con este aminoácido durante 60 min, es disminuida por la presencia de fenilalanina pero aumentada por la histidina. El efecto de la histidina se puede explicar por intercambio a nivel de la membrana entre la histidina intracelular y el triptófano extracelular.

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The Threshold for the Smell of Acetone and its Relationship to the Ability to Taste Phenylthiocarbamate[1]

The odor of acetone in the breath of patients in diabetic coma has long been thought to be of diagnostic significance. Since many experienced clinicians are unable to detect any distinctive odor in such patients[2], while others can, we investigated the smell threshold for the odor of acetone and compared it with the ability to taste phenylthiocarbamate (PTC) of the same subjects. The population studied, mostly students and clerks, included 211 subjects, 86 between 15 and 19 years of age, 65 between 20 and 39 and 60 between 40 and 69.

Nine dilutions of reagent grade acetone in deionized water were prepared in glass-stoppered test tubes 1–2 h before each test session. They ranged in concentration (vol./vol.) from 8.0 to 0.01% (legend, Figure 1). Each tube was mixed by inversion before being presented to the subject to smell. The tubes were offered in order of increasing concentration after familiarizing the subject with the smell of acetone. When the approximate threshold was reached, the corresponding tube was placed together with 3 tubes of deionized water for identification as suggested by the method of HARRIS and KALMUS[3] for determining the threshold for the taste of PTC. The lowest concentration of acetone correctly identified from among the tubes of water was taken to be the threshold.

Ability to taste PTC was determined by having the subject taste a drop of an aqueous solution of 8.125 mg PTC per 100 ml, the antimode in the distribution of PTC thresholds[3]. If the subject noted any taste at all he was considered a taster.

When the distribution of thresholds for the smell of acetone in young subjects (aged 15–39) was plotted separately for males and females, 3 definite peaks were found in each graph (Figure 1). In the graph of the older subjects (aged 40–69) there were 2 peaks and a 'shoulder', each corresponding to 1 of the 3 peaks in the graph of the younger group (males and females combined), but in the

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