chloride tent was permeable to NH_3 and that small amounts of $^{14}NH_3$ leaked into the tent. Sodium hydroxide hydrolysis of the MgO-distilled water from the tent did release small amounts of NH_3. These data suggest that small amounts of amide N may have leaked or been present in the condensed water from the plants.

Our study illustrates that growing plants are a sink for atmospheric NH_3 and can absorb considerable quantities of NH_3 from the atmosphere. Green plants, like rain, may cleanse the atmosphere of this possible N pollutant. They may also meet a portion of their N needs by absorbing atmospheric NH_3.

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30 September 1971; revised 10 November 1971

Dihydroxyphenylalanine in Rat Food Containing Wheat and Oats

Abstract. *Dopa has been identified in rat food by three different fluorimetric assays and paper chromatography. Incubation of the rat food with proteolytic enzymes dramatically increased the measurable free dopa. Analysis of samples of six individual protein-containing constituents of rat food revealed that both wheat and oats contain dopa.*

High concentrations of dopa (dihydroxyphenylalanine) (1 to 2 μg per gram of tissue) in the rat stomach have been observed in this laboratory (*1*). Administration of [^{3}H]tyrosine by stomach tube (10 μc/100 g) to starved rats did not lead to the accumulation of [^{3}H]dopa in the stomach. We therefore suspected that the gastric dopa was of dietary origin. Dopa has not generally been considered to be a dietary constituent, though its glucuronide can be extracted from broad bean, *Vicia faba* (*2*, *3*).

Solid pellets of Big Red laboratory animal chow (Agway, Syracuse, New York) were pulverized with a mortar and pestle and then suspended in water. Portions of the suspension were then analyzed directly for dopa (see Tables 1 and 2) or incubated at 37°C for 16 hours with or without the proteolytic enzyme Pronase [Calbiochem, 0.1 percent (weight to volume)] (*4*). Several drops of ethanol were added to inhibit bacterial growth. After the incubation, one volume of 30 percent trichloroacetic acid was added to four volumes of hydrolyzate, and the resulting mixture was centrifuged at 27,000*g* for 15 minutes. The supernatant was filtered through paper, and the resultant solution was diluted to 10 ml with 7.5 percent trichloroacetic acid (TCA). Portions (0.25 ml) of the diluted supernatant were set aside for the assay of tyrosine (*5*). The dopa was absorbed from the TCA supernatant onto Dowex 50W-4X and then alumina columns as described (*6*). Portions of the final acetic acid eluates from the alumina columns were assayed fluorimetrically for dopa by the trihdroxyindole reaction (*7*). The resulting activation and emission spectra for the dopa extracted from rat food correspond exactly with those of the added dopa standard (Fig. 1a). Identical fluorescence spectra were also obtained when the direct oxidative coupling reaction (*8*) was performed on the alumina eluates (Fig. 1b). When 10.0 g of the rat food was processed, native catechol fluorescence could be seen in the alumina eluate (Fig. 1c). This fluorescence could not have come from dopamine and norepinephrine since, as shown (*6*, *7*), these catecholamines are separated from the dopa by the Dowex chromatography before the alumina absorption. After lyophilization, portions of the alumina eluate were also subjected to ascending paper chromatography with the solvent system butanol, acetic acid, H_2O (4 : 1 : 1). The dried paper chromatogram was sprayed with ethylenediamine, and observed under ultraviolet light. An intense yellow fluorescence was observed with the same R_F (0.16) as that of the added dopa standard.

The free dopa concentration was dramatically increased by the inclusion of Pronase in the incubation mixture (Table 1). Pronase has esterase activity (*9*), and thus could have liberated free dopa from its glucuronide (*3*). Incubation of the rat food with β-glucuronidase H2 (Sigma Chemical) did not, however, increase the measurable dopa. We obtained evidence that the dopa was not simply present as a glucuronide by suspending the food in water, adding equal volumes of pure alcohol, and allowing the mixture to sit in ice for 1 hour to precipitate the protein. The mixture was centrifuged as described, and the supernatant was discarded. The pellet was suspended in water, the suspension was centrifuged, and the supernatant was again discarded. Any dopa-glucuronide would thus have been eliminated. The protein pellet was suspended in water a second time, and then digested with

Table 1. Dopa content of rat food. Data are given as mean ± standard error of the mean (S.E.M.); *N*, number of determinations; TCA, trichloroacetic acid.

Rat food	L-Dopa (μg/g)	Tyrosine (mg/g)	Dopa/tyrosine
TCA supernatant	0.087 ± 0.013 ($N = 4$)		
Suspension in H_2O	0.332 ($N = 2$)		
Incubation of rat-food suspension			
Without Pronase	1.29 ± 0.4 ($N = 5$)	0.175 ± 0.034 ($N = 4$)	7.4×10^{-3}
With Pronase	23.8 ± 3.9 ($N = 9$)	2.55 ± 0.35 ($N = 6$)	9.3×10^{-3}
Incubation of protein precipitate with Pronase	45 ($N = 3$)	4.1 ($N = 2$)	10.9×10^{-3}

Table 2. Dopa content of various components of rat food.

Sample	Suspended in H_2O (μg/g)	Incubated	
		Without Pronase (μg/g)	With Pronase (μg/g)
Alfalfa	0.31	0.36	0.78
Corn	0	0	0
Evaporated milk	0	0	0
Oats	.112	.321	24.8
Soybeans	0	0	0
Wheat (durum)	.136	.793	6.24

Pronase. With this technique, the yield of dopa was, if anything, slightly higher than when the Pronase digestion was performed directly on the whole rat food suspension (Table 1).

The mechanism responsible for the increase in free dopa subsequent to Pronase hydrolysis is not yet clear. The dopa is not being formed by a direct action of Pronase, inasmuch as incubation of tyrosine (1 mg) or hemoglobin (5 mg), a protein of known composition, with Pronase failed to cause the appearance of dopa fluorescence. When tyrosine was incubated with the rat food suspension, in the absence of Pronase, approximately 2.0 percent of the tyrosine was converted to dopa. This conversion could be blocked by boiling the rat food for 3 minutes prior to the incubation, suggesting that it was catalyzed by a tyrosine-hydroxylating enzyme present in the food. Hence it is possible that some or all of the increase in free dopa that occurs after Pronase digestion results from the hydroxylation of free tyrosine liberated from peptide chains. This increase in free dopa could also represent the dopa which is a constituent of the peptide chain in the rat food protein. It is well known that the peptide chains of collagen and myosin contain other modified amino acids, hydroxyproline, and methylhistidine, respectively (*10, 11*). However, since free dopa is generated from free tyrosine during the Pronase digestion, we hesitate to accept the Pronase effect as evidence for or against the peptide linkage of dopa. Chemical hydrolysis of the protein in rat food led to the destruction of added dopa, and presumably of any dopa that might have been in peptide linkage.

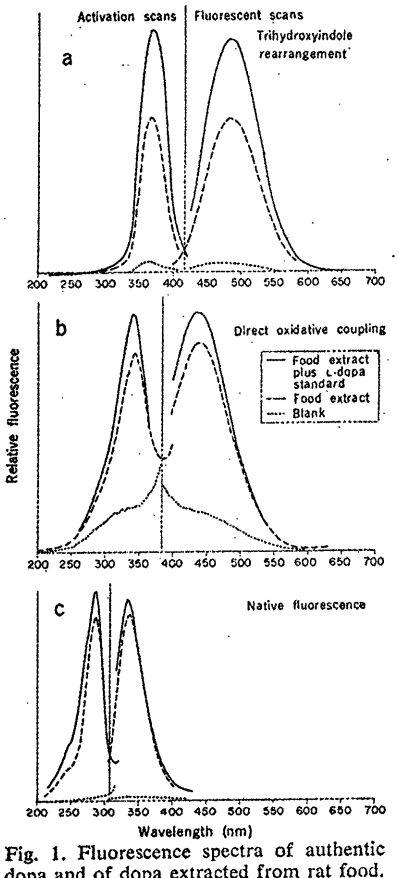

Fig. 1. Fluorescence spectra of authentic dopa and of dopa extracted from rat food. (a and b) Spectra obtained after oxidation by the trihydroxyindole reaction (*7*) and the direct oxidative coupling reaction (*8*). (c) Excitation and emission spectra of the native catechol.

The dopa concentrations in the six major constituents of the rat food can be seen in Table 2. The cereals were obtained from the Erewhon Trading Company, Boston, Massachusetts. Proteins were precipitated with TCA after Pronase hydrolysis, as described. Dopa was measured by the trihydroxyindole rearrangement (*7*), our most sensitive assay. Oats and wheat were found to be major sources of dopa.

The presence of dopa in cereals provides a reasonable explanation for the occurrence of dopamine in the gastrointestinal tract and liver of ruminants (*12, 13*). The dopamine was probably derived from the dopa contained in cereal proteins they ingested. The absence of dopa in evaporated milk (Table 2) is equally interesting. Weil-Malherbe and Van Buren reported a decrease in urinary dopamine excretion among adult subjects ingesting a diet consisting primarily of evaporated milk (Lactum baby food) (*14*). We have reported a decrease in free urinary dopamine in children in the acute phase of kwashiorkor (*15*); these children were being fed all-milk diets. Friedhoff and Coupet have observed a decrease in the homovanillic acid excretion of rats fed an all-glucose diet (*16*). These observations suggest that the dopamine and its metabolites normally present in urine reflect, in part, dietary dopa. The same conclusion may pertain for urinary 3,4-dimethoxyphenylethylamine (DMPEA). The excretion of this compound has been observed to decrease following the administration of an all-glucose diet (*17*) or plant-free diet (*18*). It thus seems reasonable to suspect that this "abnormal" urinary constituent is also derived from dietary dopa and may have no specific relation to psychiatric disease (*19*).

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20. Supported in part by grants from the Barra Foundation, the Penick Foundation, and U.S. Public Health Service (AM-14228), and by PHS training grant AM-05371 to R.H. We thank Dr. Steven Tannenbaum for helpful suggestions. This report is contribution No. 1861 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology.

1 September 1971; revised 20 October 1971