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SELECTIVE EFFECT OF A MAIZE DIET IN REDUCING SERUM AND BRAIN TRYPTOPHAN CONTENTS AND BLOOD AND BRAIN SEROTONIN LEVELS

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Summary

The Maize diet used selectively lowers the tryptophan, serotonin and 5-hydroxyindoleacetic acid levels in the central nervous system without affecting catecholamine content. These changes are maximal as early as 48 hours after exposure to the Maize diet. The brain serotonin decrease is reversed to normal when the Maize diet is supplemented with Tryptophan, while nicotinic acid is ineffective. The maize diet seems to be a rapid and selective means of reducing brain serotonin content. The hypothesis that the psychic depression observed in patients with Pellagra may be related to an altered serotonin metabolism is discussed.

A large body of evidence suggests that diet may affect the functional activity of the brain and a tight relation between "nutrition and mind" has been proposed (1). Therefore, nutrition is now recognized to play an important role in explaining some behavioral changes that occur in animals and man.

Tagliamonte et al. (2) and Knott and Curzon (3) have recently clarified the function of serum free tryptophan (TP) levels in regulating whole brain TP content. Since TP is the serotonin (5-HT) precursor, any variation in its free level in serum affects the biosynthesis of the amine. Tagliamonte et al. (4,5) have also underlined the importance of the existing equilibrium between plasma and brain TP in regulating 5-HT biosynthesis in brain neurons.

Fernstrom and Wurtman (6) showed that a chronic consumption (5 weeks) of a dry corn diet, a staple deficient in TP and containing nicotinic acid in a bound form (7), depressed the brain 5-HT levels in rats.

A large number of people subsist on corn diets as their major protein source. A diet high in Maize is known to cause Pellagra, a typical nutritional disease (7).

For many years, the nicotinic acid deficiency was thought to be responsible for the Pellagra syndrome, and only recently have TP deficiency and an altered 5-HT metabolism been postulated as responsible for the mental disorders (insomnia, depression, emotional instability, disorientation) accompanying this disease (8). Consistent with this hypothesis is the significantly low 5-HT concentration found in platelets of Pellagra patients with mental depression (9).

To further explore the effect of a deficient intake of TP on the serotoninergic system, 5-HT metabolism was examined in young adult rats fed from 2 up till 20 days a Maize diet provided in a cooked mush.

Materials and Methods

Male Sprague-Dawley rats with an initial weight of 180 g were used. They were housed 5 per cage and had free access to food and water.

The tryptophan-deficient diet consisted of a mush of Maize meal cooked in salted water for 40 min (1 kg of Maize/4 l of water). The tryptophan-sufficient diet was prepared by supplementing 1 kg of the above diet with 2.5 g of L-tryptophan. The control groups were fed Charles River pellets (normal diet).

To avoid other possible dietary deficiences, expecially that of nicotinic acid, the drinking water was enriched by addition of nicotinic acid (3 mg), Vit. B_1 (0.26 mg), Vit. B_2 (0.26 mg), Vit. B_6 (0.13 mg), Vit. E (0.26 mg) Vit. C (83 mg), Pantothenic acid (0.26 mg), Vit. A (1380 IU) and Vit. D_2 (266 IU) per 100 ml of water.

The experiment was carried out over 20 days, with groups of animals killed on day 2, day 7, day 15 and day 20. Rats were killed by decapitation, always in the afternoon. Brain, minus cerebellum, was quickly removed, frozen on dry ice and stored at -20°C until analyzed. Blood was collected in tubes containing Heparin and plasma was obtained by centrifuging at 2000 rpm for 10 minutes.

Brain was homogenized with 10 volumes of acidified butanol according to the procedure of Maickel et al. (10). The resulting homogenate was centrifuged at 3000 rpm for 10 min and the butanol phase was used for 5-HT and 5-hydroxyindolacetic acid (5-HIAA) determinations by the method of Curzon and Green (11). A modification of the method described by Spano et al. (12) was used for plasma and brain TP assay. The amino acids were extracted into 20 volumes of acidified butanol and re-extracted into 2 ml of 0.1 N HCl after adding 2 volumes of heptane to the butanol phase. The acid phase was adjusted to pH 4.0-4.5 with 0.1 M sodium acetate buffer pH 6, and passed through a Dowex 50 x 4 column prepared as described by Haggendal (13). Tyrosine (Tyr) was eluted with 0.1 M sodium acetate buffer pH 4.5, according to Neff et al. (14) and flurimetrically assayed as described by Underfriend (15). TP was eluted with 0.1 M sodium acetate buffer pH 6 and assayed by the method of Denkla and Dewey (16). Blood 5-HT was measured in whole blood collected in tubes containing 15% EDTA and 3% ascorbic acid pH 6 and immediately frozen until assayed. 5-HT was then determined according to the method suggested by Geeraerts et al. (17). Brain norepinephrine (NE) and dopamine (DA) were assayed as described by Brodie et al. (18).

Results

The rapid and significant decreases with time of brain TP and 5-HT and of spinal cord 5-HT following exposure to the Maize diet are shown in Table 1. In contrast, during the time period examined the levels of brain Tyr, NE and DA remained constant (Table 2). Similarly, a significant decrease in total plasma TP levels and in the whole blood 5-HT content was observed, while plasma Tyr concentration remained unmodified (Table 3). The brain and spinal cord 5-HT decrease was reversed to normal when the Maize diet was supplemented with TP (Table 4). Similar results were obtained when the Maize diet was replaced for two days with Charles River pellets, although the brain 5-HT levels were still slightly below the normal (Table 4).

TABLE 1

Effect of a Maize diet on brain tryptophan (TP), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and on spinal cord 5-HT levels.

Days on diet	BRAIN			SPINAL CORD
	TP	5-HT	5-HIAA	5-HT
2	47.72±5.71*	69.26±1.56*	61.32±3.70*	70.69±2.44*
7	59.68±6.20*	68.09±3.96*	57.51±5.53*	74.82±4.57*
15	42.42±7.13*	72.06±2.67*	47.57±3.98*	75.01±4.64*
20	52.32±8.00*	67.59±6.76*	49.98±5.42*	---

The figures indicate mean values ± SE for 10 animals per group and are expressed as percentages of control values (= 100). * $p < 0.001$
Average of control values for all time periods (25 animals): TP = 3.58 ± 0.23 µg/g frozen brain; 5-HT = 0.71 ± 0.01 µg/g frozen brain and 0.84 ± 0.02 µg/g frozen spinal cord; 5-HIAA = 0.55 ± 0.01 ug/g frozen brain.

TABLE 2

Effect of a Maize diet on brain tyrosine (Tyr), norepinephrine (NE) and dopamine (DA) levels.

Days on diet	Tyr µg/g ± SE		NE µg/g ± SE		DA µg/g ± SE	
	Normal	Maize	Normal	Maize	Normal	Maize
2	15.5±2.4	13.8±1.3	0.59±0.01	0.62±0.03	1.25±0.02	1.27±0.04
7	13.4±0.6	10.9±0.9	0.60±0.02	0.61±0.02	1.28±0.03	1.29±0.06
20	---	---	0.56±0.02	0.52±0.01	1.34±0.02	1.26±0.05

Values represent the mean ± SE for 6 animals per group.

TABLE 3

Effect of a Maize diet on total plasma tryptophan (TP) and tyrosine (Tyr) and on blood 5-hydroxytryptamine (5-HT) levels.

Days on Maize diet	PLASMA Tyr	PLASMA TP	BLOOD 5-HT
2	80.12 ± 8.80	40.08 ± 6.36**	---
7	90.07 ± 9.96	47.00 ± 9.81*	56.29 ± 1.70**

The figures indicate mean ± SE for 10 animals per group and are expressed as percentages of control values (= 100). **$p < 0.001$ *$p < 0.01$
Control values (average of 20 animals): TP = 16.29 ± 1.58 µg/ml plasma; 5-HT = 1.35 ± 0.06 µg/ml whole blood; Tyr = 13.54 ± 1.94 µg/ml plasma.

TABLE 4

Effect of a tryptophan supplemented Maize diet on brain and spinal cord 5-hydroxytryptamine (5-HT) levels.

Experiment	5-HT µg/g ± SE	
	BRAIN	SPINAL CORD
17 days Charles River pellets	0.789 ± 0.019	0.753 ± 0.14
17 days Maize diet	0.540 ± 0.029**	0.568 ± 0.03**
15 days Maize diet + 2 days tryptophan sufficient diet	0.770 ± 0.031	0.726 ± 0.01
15 days Maize diet + 2 days Charles River pellets	0.703 ± 0.037*	0.721 ± 0.02

Values represent the mean ± SE for 10 animals per group.
**$p < 0.001$ *$p < 0.05$

Nicotinic acid added to the diet failed to affect the 5-HT and 5-HIAA brain levels in rats fed either standard laboratory pellets or Maize diet (Table 5).

TABLE 5

Effect of Vitamins added to the drinking water on brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels of rats fed a normal or a Maize diet during a 7 days period.

Experiment	5-HT µg/g ± SE	5-HIAA µg/g ± SE
Charles River pellets	0.68 ± 0.03	0.60 ± 0.01
Charles River pellets + Vitamins	0.67 ± 0.01	---
Maize diet	0.50 ± 0.01*	0.39 ± 0.01*
Maize diet + Vitamins	0.48 ± 0.01*	0.39 ± 0.01*

Values represent the mean ± SE for 10 animals per group.
*$p < 0.001$
For experimental details see Material and Methods

During all the experimental period, rats fed Maize diet remained at significantly lower body weight than those fed normal diet (Fig. 1).

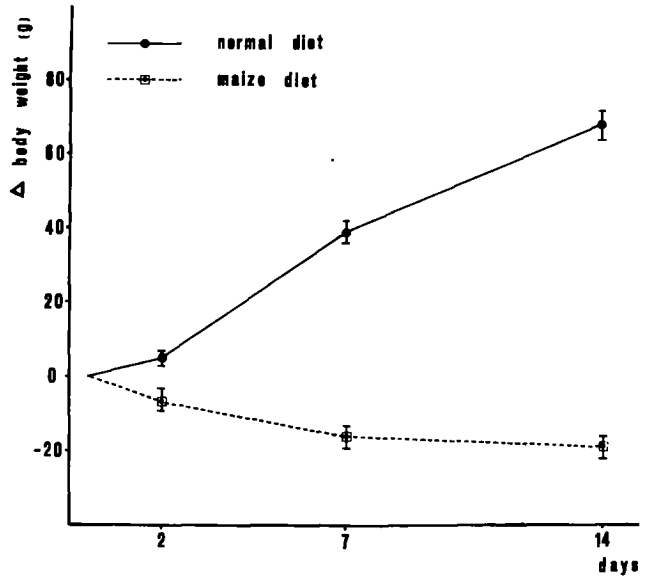

FIG. 1 - Effect of a Maize diet on the growth curve of rats.

Although no behavioral measurements were performed, the animals consuming the Maize diet showed evident signs of excitability and aggressiveness, which were most evident during the third week of observation.

DISCUSSION

The data reported in this paper suggest that a Maize diet rapidly and selectively decreases the central nervous system 5-HT and 5-HIAA levels. This effect is paralleled by the TP decrease in both plasma and in brain, and is completely reversed by adding TP to the diet, while addition of nicotinic acid was ineffective.

Since the changes in TP, 5-HT and 5-HIAA brain levels were selective and occurred as early as 48 hours after exposure to the Maize diet, it seems reasonable to conclude that the data obtained are not due to a non specific nutritional deficiency, but to the low TP content of the Maize diet.

Similar results for brain indole levels have been reported by Fernstrom and Wurtman (6) in rats fed a dry corn diet for 5 weeks. These results, however, did not report any data on the time course of brain TP and 5-HT depletion.

Brain TP and 5-HT levels have been shown to depend on free plasma TP (2, 3, 5) or, alternatively upon total plasma TP in competition with other neutral amino acids (19, 20) - such as leucine - which compete for the same transport mechanism from blood to brain.

The excess of leucine in the Maize diet (21), by competing with the already low TP levels, might thus partecipate in the lowering of brain TP and 5-HT levels.

As pointed out above, the brain, spinal cord and blood 5-HT decreases occur quickly, as early as 48 hour after exposure to the diet. These findings are similar to those reported by Culley (22), who provided evidence, using a synthetic deficient diet, that plasma TP and brain 5-HT levels were both significantly lowered by the fourth day of dieting; these changes were then stabilized for the following 24 days of observation. Therefore, the Maize diet may be a simple, inexpensive tool, that could substitute for the more complex synthetic diet to induce a TP deprivation in rats.

It seems worth noting that this diet induces a specific and selective effect on the serotoninergic neurons, since it does not affect the catecholamine system. The selective action of this diet may be a valuable alternative to the well known pharmacological methods such as p-chlorophenylalanine (23) and 5,6-dihydroxytryptamine (24) administration used for reducing brain 5-HT content.

Finally, since a Maize diet induces Pellagra, emphasis should be placed on the evidence in support of the hypothesis that TP deficiency may be associated with promoting the mental changes which accompany this syndrome. The above data support Krishnaswamy's hypothesis (8) that psychic depression observed in Pellagra patients may be related to an altered serotonin metabolism and that it might be improved by TP administration.

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