Effect of a Tryptophan Deficient Diet on Brain Serotonin and Plasma Tryptophan Level.* (28451)

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(Introduced by R. C. Corley)
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It has been well established through the use of tracers that tryptophan, an essential amino acid, is a necessary precursor of serotonin for animals(1). The variation of brain serotonin level with the level of tryptophan in the diet has been demonstrated several times. For instance, brain serotonin level increases substantially when tryptophan supplements are added to diets(2,3) and, conversely, a 37% decrease in brain serotonin was noted after rats had been fed a tryptophan deficient diet for over 4 weeks(4). Others(5) demonstrated a decrease of approximately 65% after 5-6 weeks on a tryptophan deficient diet. However, Green *et al.* (2) found no significant decrease in brain serotonin of young white rats placed on a tryptophan low diet for 14 to 17 days. These previous studies have not included measurements of brain serotonin level as a function of time on a tryptophan deficient diet. Also the change in plasma tryptophan level with respect to brain serotonin level has not been explored. These two points are the basis for this investigation.

Methods and materials. One hundred and eight female Wistar rats weighing approximately 165 g each were divided into 2 groups. One group received a .5% L-tryptophan supplement in the diet and the other received the unsupplemented diet, the composition of which was as follows: acid-hydrolyzed casein, salt free powder (NBCo.)—20%; glucose—47.7%; cornstarch—20%; celluflour—2%; salt mix, U.S.P. XIV—5%; DL-methionine—.3%; Wesson oil—5%. The following amounts of vitamins were added to 8 kg of this feed: thiamin hydrochloride—80 mg; riboflavin—80 mg; pyridoxine hydrochloride—80 mg; choline chloride—16 g; calcium pantothenate—320 mg; nicotinamide—16 g; p-aminobenzoic acid—1.6 g; inositol—4 g; folic acid—16 mg; biotin—2 mg; Vit. B_{12}—1 mg; Vit. A—200,000 units; Vit. D_2—25,000 units; alpha-tocopherol—2000 units and menadione—160 mg. All animals were housed in wire bottom cages and were fed and watered *ad libitum*. For at least 2 weeks before being placed on experiment, all animals received the above diet except that casein was substituted for casein hydrolysate and the nicotinamide level was 80 mg per kg of feed.

At the appropriate times the animals were anesthetized with ether and heparinized cardiac blood was drawn for tryptophan determinations. The skull was then immediately opened and the whole brain removed and immediately homogenized in 2 parts .1 N hydrochloric acid. The blood was centrifuged and plasma tryptophan level determined(6). Whole brain serotonin was measured by the method of Bogdanski(7). Six animals from each group were sacrificed after 2 days, 4 days and every multiple of 4 days until the experiment was terminated.

Results and discussion. Table I shows that plasma tryptophan and brain serotonin are both depressed significantly ($P<.01$) after as little as 4 days on this tryptophan deficient diet. However, the level of plasma tryptophan, 35% of normal, is more severely depressed after 4 days than the brain serotonin level, 64% of normal. Previous reports(4,5) found significant depressions of brain serotonin after more than 4 or 5 weeks on tryptophan deficient diets but no reports have indicated a depression after as little as 4 days. It is interesting to note that the brain serotonin level does not steadily decrease with time spent on the tryptophan deficient diet but rather stabilizes at the level noted after 4 days on diet, 65% of nor-

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TABLE I. Plasma Tryptophan and Brain Serotonin Levels.

Days on diet and treatment	Body wt (g) At start	Body wt (g) When sacrificed*	Plasma tryptophan (mg/100 cc)	Plasma tryptophan (%)	Brain serotonin (μg/g wet wt)	Brain serotonin (%)
2 (Controls)	170		1.9		.51	
2 (Tryptophan deficient)	167		1.6	(84)†	.49	(96)
4 (Controls)	160		1.7		.54	
4 (Tryptophan deficient)	151		.6	(35)	.35	(64)
8 (Controls)	168	180	1.9		.53	
8 (Tryptophan deficient)	167	148	.8	(42)	.34	(65)
12 (Controls)	168		1.8		.50	
12 (Tryptophan deficient)	166		.7	(39)	.34	(69)
16 (Controls)	170	190	2.1		.52	
16 (Tryptophan deficient)	169	130	.8	(38)	.37	(72)
20 (Controls)	163		1.8		.55	
20 (Tryptophan deficient)	165		.6	(33)	.32	(59)
24 (Controls)	165	192	2.0		.54	
24 (Tryptophan deficient)	160	114	.7	(35)	.32	(60)
28 (Controls)	167		1.8		.54	
28 (Tryptophan deficient)	167		.25	(14)	.17	(32)
32 (Controls)	166	196	2.1		.51	
32 (Tryptophan deficient)	160	110	.35	(16)	.18	(36)

* Final body weights were determined only on animals sacrificed at the end of 8, 16, 24 and 32 days.

† All numbers in parentheses indicate experimental value as percentage of control value.

mal, and remains near that figure until 28 days on diet when there is a rather abrupt further decrease ($P<.01$) to the neighborhood of 30-35% of normal. This latter level is comparable to that noted by Gal and Drewes(5) for rats of this size after 5-6 weeks on a tryptophan deficient diet. Their feeding differed from ours, however, in that their diet contained 15.6% casein hydrolysate and 1.0% DL-tryptophan and was fed at the controlled rate of 7 g per day. Also, in contrast to that study, none of our animals died during the experiment even though the diet contained no sulfas or antibiotics. The fact that our animals were fed *ad libitum* a diet of different composition may account for this. However, our experimental animals on the tryptophan deficient diet 24 and 32 days lost a greater percentage of their body weight than did those on the Gal and Drewes diet. The reason for the sub-optimal weight gain of the control animals (Table I) is not immediately apparent. The very high level of nicotinamide in the diet in addition to the .5% L-tryptophan supplement may have been a factor. Our findings are not in agreement with a previous report(8) that brain serotonin level of 150 g male Sprague-Dawley rats was depressed less than 5% after being on a tryptophan deficient diet for 10 days but was depressed approximately 25% after 17 days on diet. However, the fact that those rats were of a different strain, were males rather than females, and were fed a slightly different diet may explain why in their experiment such a small depression in brain serotonin was noted in 10 days while in this experiment significant depressions were noted after 4, 8 and 12 days on diet.

It has been demonstrated(9) that inanition does not affect brain serotonin level which is evidence that the weight loss of the tryptophan deficient animals is not contributing to their decrease in brain serotonin.

There was a highly significant correlation ($r \equiv .87$, $P<.01$) between the plasma tryptophan levels and brain serotonin levels of the groups of experimental rats sacrificed at the indicated times. Although the plasma tryptophan level of the experimental animals reliably indicated the times at which brain serotonin level changed, the magnitude of change expressed as percent of normal was greater for plasma tryptophan than for brain serotonin. It could be speculated from these data that a diet sub-optimal in but not devoid of tryptophan, regulated so as to lower plasma tryptophan less drastically, might not affect brain serotonin level. As men-

tioned earlier, Green *et al.*(2) fed a diet to weanling rats containing two-fifths the level of tryptophan in the control diet and no significant change in brain serotonin was noted. Plasma tryptophan was not measured.

The reason for the stabilization of the plasma tryptophan and brain serotonin level from 4 until 24-28 days on diet is not understood although probably the breakdown of tissue protein was maintaining the plasma tryptophan at this reduced but constant level until these stores were depleted, when the further decrease noted at 28 days occurred.

The relatively rapid lowering of brain serotonin in rats fed a tryptophan deficient diet is of interest from the standpoint of mental function. It is well known that blood serotonin is depressed in human phenylketonuria (10) and probably brain serotonin is also lowered. In fact, the lowering of brain serotonin in rats with simulated phenylketonuria has been noted by several investigators(3,11, 12,13). This has frequently been mentioned as possibly the causative factor in the retarded learning ability typical of phenylketonuria. However, Louttit(11) noted that rats on high phenylalanine diet performed no more efficiently on the Hebb-Williams Maze when a monoamine oxidase inhibitor was added to the diet in an amount that raised the brain serotonin to normal or above normal levels. Others(12) found that adding a tryptophan supplement to the diet of rats fed high phenylalanine levels increased their ability to run a water maze. They noted, however, that the serum phenylalanine level decreased after adding the tryptophan supplement so factors other than serotonin level could have accounted for this change.

It has recently been shown(14) that lowering brain serotonin in adult mice for even brief periods increased their maze learning ability and increasing brain serotonin level decreased maze learning abilities of adult mice. However, the authors reported that brain serotonin depression early in life decreased learning ability. In that case, tryptophan deficiency early in life should reduce learning ability and tryptophan deficiency in adults should increase learning ability unless other factors involved in tryptophan deficiency mask such an effect. Such findings would be of interest to people in parts of the world where infants and children are fed tryptophan poor diets. Studies on the effect of tryptophan deficient diets on maze learning ability of young and adult rats are in progress.

In view of these results, it seems reasonable to conclude that when rats are placed on low tryptophan diets, changes in plasma tryptophan level reflect changes in brain serotonin level although the magnitude of these changes is not comparable. Furthermore, brain serotonin is significantly decreased after as little as 4 days on such diets, then stabilizes until at 28 days on diet it decreases further.

Summary. One hundred and eight female Wistar rats were placed on tryptophan sufficient or tryptophan deficient diets. Six animals from each group were sacrificed after 2 days, 4 days and multiples of 4 days. Plasma tryptophan level was found to reflect changes in brain serotonin level. Furthermore, plasma tryptophan and brain serotonin were significantly decreased after as little as 4 days on the tryptophan deficient diet. The levels of plasma tryptophan and brain serotonin were relatively constant from 4 to 28 days, at which time both showed further decreases.

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Studies on the Binding of Ascorbic Acid in Animal Tissues.* (28452)

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Ascorbic acid is highly soluble in water and diffuses readily through tissue membranes. It has the physical characteristics of a monosaccharide but it is not distributed uniformly in tissues like glucose and there is no known polymerized storage form in tissues comparable to glycogen.

The concentration of ascorbic acid in the various tissues in the animal body varies widely. This is shown by Table I prepared from the data of Kuether, Telford and Roe (1). The tissue levels of ascorbic acid in guinea pigs upon a high intake of ascorbic acid (cabbage *ad lib*) were found to range from 3 to 157 times the blood level. These data indicate that there is some mechanism that maintains storage of ascorbic acid in animal tissues.

To account for the storage of ascorbic acid it has been assumed that this compound exists in tissues bound to protein. Evidence for this assumption was obtained by Sumerwell and Sealock(2). These authors homogenized liver under 95% ethyl alcohol, centrifuged the mixture, decanted the supernatant and washed the residue 5 times with 95% ethyl alcohol. The washed residue was hydrolyzed by boiling under 5% metaphosphoric acid for 30 minutes. Free ascorbic acid was always found in the hydrolysate. Sumerwell and Sealock found, per gram of pork liver, 80 μg of ascorbic acid in the supernatant and 18 μg in the washed residue. They concluded that the ascorbic acid obtained from the washed liver residue probably existed in a form bound to protein.

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Experimental. We repeated the procedure of Sumerwell and Sealock except that a high speed homogenizer was used instead of a Waring Blendor. Rat liver was ground under 95% ethyl alcohol in a Virtis homogenizer with homogenizing beads for 5 minutes at 45,000 rpm. The mixture was centrifuged, the supernatant was decanted and the procedure of grinding and washing was repeated upon the residue 4 times. The residue was suspended in 5% metaphosphoric acid solution and boiled for 30 minutes. The ascorbic acid in the hydrolysate was determined by the method of Roe and Kuether(3). Results are shown in Table II. After 5 homogenizations and washings, there still remained in

TABLE I. Distribution of Ascorbic Acid in Tissues of Guinea Pigs Upon a High Intake of Ascorbic Acid (Cabbage *ad lib*).

	Concentration of ascorbic acid	
	Mg per 100 g or ml	Tissue level/ Whole blood level
Whole blood	.75	—
Muscle	2.39	3.1
Heart	7.54	10.0
Kidney	9.14	12.1
Brain	18.60	24.6
Liver	23.77	31.5
Spleen	42.83	56.8
Adrenal	118.60	157.3

TABLE II. Ascorbic Acid in Residue of Liver Homogenate After 5 Homogenizations and Washings with 95% Ethyl Alcohol.

	Mg ascorbic acid per 100 g		
	Total	Residue after 5 washings	% of total in residue
Liver 1	37.0	.7	1.9
" 2	29.4	.7	2.5