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Effect of Dietary Phenylalanine and Tryptophan on Pineal and Hypothalamic Serotonin Levels.* (28779)

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Serotonin (5-hydroxytryptamine) appears to be significant in normal brain function since its formation, levels and metabolism within the brain have been experimentally related to certain brain activities or their behavioral manifestations. Reduction in brain serotonin in rats occurs with a tryptophan-deficient diet(1) or increased dietary L-phenylalanine(2,3,4,5), and increase occurs with an increased intake of L-tryptophan(2). It is important, however, to examine these changes in anatomically and functionally discrete areas of the central nervous system, since their occurrence may not be general and their further physiologic and behavioral effects may depend upon such regional differences. The hypothalamus was selected for examination since its serotonin content is known to be among the highest of mammalian brain areas and since its content of, and suspected regulation by, neurohumoral substances is of great interest. The epiphysis cerebri or pineal organ, a probably glandular derivative of the central nervous system, has the greatest concentration/weight of serotonin(6) and has a distinctive serotonin metabolism, leading via hydroxyindole-O-methyl transferase activity to formation of melatonin (7,8) and possibly other compounds.

Methods and materials. Four-week-old, Long-Evans, male rats, born and raised in the same suite of windowless, artificially lighted (14 hours/day) rooms were used in replicate experimental series in which siblings were distributed between their original litter cage with standard laboratory chow and solid floor and sides (= cage controls) and individual suspended wire mesh cages (Bussey type) containing a powdered, standard, controlled diet mixture without amino acid supplement (= diet controls), or the same diet containing 7% L-phenylalanine, or the same diet containing instead 5% L-tryptophan. The standard diet was 20% ground gluten, 76% ground whole yellow maize, 3% calcium carbonate, and 1% sodium chloride. Complete vitamin supplement, including viosterol, was mixed with this. Animals were weighed at the start and at termination of the experiments and were fed *ad libitum.*

All animals were sacrificed by decapitation during the mid afternoon of the daily light period because this is the period of relatively high level and relatively greatest stability in the daily rhythm in pineal serotonin content(9). A daily rhythm in rats' hypothalamic serotonin content does not seem to occur(9). Animals of diverse categories of age, diets and lengths of treatment were sacrificed in rough alternation. The hypothalamus was dissected out by means of 5 cuts with a razor blade through: first, transverse planes at the posterior edge of the optic chiasma, and the anterior limit of the mammillary nuclei; then, lateral planes extending dorso-laterally toward the medial edges of the caudate nuclei (cauda) from the ventral furrow on the lateral edges of the

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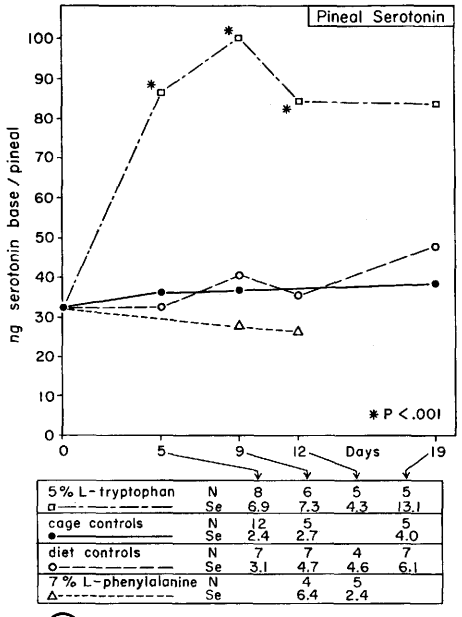
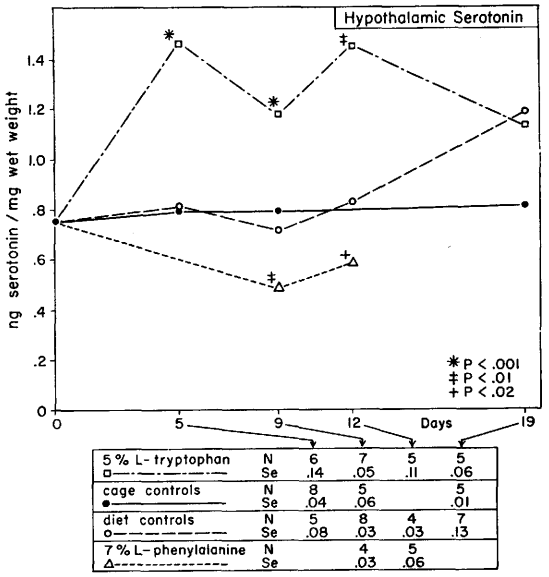


(1)



(2)

FIG. 1. Changes in pineal serotonin (graphs of means) with dietary supplements.
FIG. 2. Changes in hypothalamic serotonin (graphs of means) with dietary supplements.

hypothalamus; and finally, a dorsal plane through the ventral edge of the mammillothalamic tract. These planes were selected for ease of reproducibility, and consequently excluded extreme anterior and posterior parts of the hypothalamus. The hypothalamic blocks thus obtained were weighed to the nearest μg on aluminum foil pans with a Cahn Electrobalance. Pineal and hypothalamus of each animal were homogenized in separate 1 ml volumes of cold 0.1 N HCl (DuPont) 0.5% l-ascorbic acid (Eastman) within 1 and 5 minutes of sacrifice, respectively, and refrigerated until extraction on the following day. Extraction and fluorometric determination of the serotonin content followed a previously described procedure which excluded other 5-hydroxy and 5-methoxy indoles known to occur in these tissues(10).

Results and discussion. The effects of increased dietary tryptophan and phenylalanine on pineal and hypothalamic serotonin contents are summarized in Fig. 1 and 2. The tryptophan supplement led to a 250% increase in pineal serotonin and a nearly 100% increase in hypothalamic serotonin, both highly significant ($P < .001$). The per cent increase in hypothalamic serotonin shown here with 5% tryptophan after 5 weeks is nearly twice as great as that obtained by Wang, Harwalker and Waisman(2) in whole brain in 3 weeks starting with 18-day-old rats. The fluctuations in hypothalamic serotonin shown on days 9, 12 and 19 (Fig. 2) of the animals receiving standard powdered diets in the suspended wire mesh cages are probably related to the fact that in all of these animals some loss of weight and symptoms of irritability and stress occurred. It is suggested also, that the increased ($P < .05$) hypothalamic serotonin of diet controls on day 19 in comparison with cage controls has a similar basis. This suggestion of increased hypothalamic serotonin with weight loss and/or stress requires further experimental analysis for substantiation. Pineal serotonin content of these same animals shows less if any response of this sort (Fig. 1).

The phenylalanine supplement led to a roughly 30% reduction in both pineal and hypothalamic serotonin. If results from animals sacrificed after 9 and 12 days on the diet are pooled the significance of the depres-

sion in serotonin content is $P < .001$ for hypothalamus and $P < .02$ for pineal. The per cent decrease in pineal and hypothalamic serotonin shown here is comparable to that (ca 33%) obtained by Culley *et al*(5) in which whole brain of older rats (average weight at start of experiment = 170-185 g) on 7% L-phenylalanine for approximately 2 weeks. But the decrease is less than that (ca 75%) obtained by Wang *et al*(2) for younger rats (started when 18 days old) on this regimen for 3 weeks. No animals on the 7% phenylalanine diet were continued for 19 days due to the cost of the supplement and the fact that the 9- and 12-day results were equivalent.

Although the demonstrated increase in tissue serotonin from increased dietary tryptophan might be expected since the latter is the precursor, the magnitude of the change, especially in the pineal organ, is remarkable in the light of the presumed biological significance of the compound. In the pineal organ, where at least one specific pathway is responsible for further modification of the indole amine molecule (as to melatonin), it will be of interest to determine the qualitative and quantitative effects of afternoon serotonin level on its pineal derivatives. Unfortunately the pineal 5-hydroxy and 5-methoxy indoles other than serotonin are present in much lower quantities(10) and require pooling of many organs for accurate measurements. The low content of these may be due in large part possibly either to their rapid release or further metabolism.

The way in which phenylalanine lowers brain serotonin concentration may include partial inhibition of enzymatic formation of serotonin from tryptophan, at the hydroxylation of tryptophan(11,12) or the decarboxylation of 5-hydroxy tryptophan to form serotonin(2,11,13). However, this inhibition by phenylalanine is not great(2) and that of some of its metabolites ranges from little to none(5). A probably more significant mechanism is the demonstrated inhibition of transport of 5-hydroxytryptophan into brain by phenylalanine; and this transport may be competitive in nature and independent of the decarboxylase activity within the brain(4). Although the pineal contains 5-hydroxy tryptophan decarboxylase(14) it lacks tryptophan hydroxylase(14,15) and may be different in its uptake rate of 5-hydroxy tryptophan(16). Nevertheless, the effectiveness of the phenylalanine-induced reduction of serotonin content is equivalent to that shown in the hypothalamus and brain, and the increased serotonin store induced by high tryptophan intake is much greater than that in brain or hypothalamus.

Summary. Long-Evans rats were placed on diets containing 5% L-tryptophan or 7% L-phenylalanine or without supplements, when 4 weeks old and were sacrificed 5, 9, 12, or 19 days later. The high tryptophan diet led to a 250% increase in pineal and a nearly 100% increase in hypothalamic serotonin within 5 to 9 days. The high phenylalanine diet led to a 30% reduction in serotonin content in both tissues. These results are compared with those from whole brain.

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SV-40 Induced Proliferation of Tissue Culture Cells of Rabbit, Mouse, and Porcine Origin. (28780)

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The simian vacuolating virus (SV-40) is oncogenic for the newborn hamster(1,2) and induces transformation of hamster(3-6) and human(7-9) tissue maintained *in vitro.* Although several attempts have been made, no oncogenic action of this agent has been demonstrated in the mouse or rabbit(10,11).

To obtain more information about the relationship between transformation *in vitro* and oncogenesis *in vivo,* the effect of SV-40 infection of rabbit and murine, as well as porcine kidney monolayer cultures was studied. In all 3 types of culture a proliferative response occurred which has many similarities to the transformations described with both hamster and human tissue. In this report, transformation is defined as development, in a tissue culture, of a population of rapidly proliferating cells with altered morphology and abnormal growth pattern, with the cells maintaining their abnormal characteristics during prolonged sub-cultivation *in vitro.*

Materials and methods. Virus. SV-40 strain 777 was obtained from Dr. P. Gerber. It was isolated in African green monkey kidney (AGMK) cultures, passed once in the rhesus kidney cell line $LLCMK_2$(12), and thereafter in AGMK. The virus pools used in these experiments were from the 4th and 5th additional AGMK passages, and had infectivity titers of $10^{4.8}$ and $10^{5.3}$ $TCID_{50}$/0.1 ml, respectively. *Tissue Culture.* Cultures were prepared on contract by Dr. T. G. Ward of Microbiological Associates, Inc., Bethesda, Md.; they were prepared by trypsinization of minced kidney fragments and were grown in Eagle's basal medium (BME) with 5% calf serum and antibiotics. Rabbit kidney cultures were prepared from 2-week-old New Zealand white rabbits (Romar Rabbitry, Suitland, Md.). Mouse kidney cultures were prepared from NIH Swiss and C3H/Mai weanling mice. The latter strain is of Bittner origin and was obtained from Dr. Ludwik Gross. Porcine kidney monolayer cultures were derived from embryos.

Tube cultures were used for transformation studies when the cell sheet was fully developed. Each culture received 0.1 ml of undiluted virus; uninoculated cultures served as controls. Maintenance medium for the first rabbit kidney experiment consisted of BME with 5% heated (56°C 30 min.) agammaglobulinic calf serum,* 100 units penicillin, and 100 μg streptomycin. Thereafter, medium NCTC 109 with 10% unheated agammaglobulinic calf serum and antibiotics was used as maintenance medium for all experiments, and as growth medium for cell lines; this medium has been found particularly suitable for rapid transformation of hamster kidney cultures by SV-40(13). Cultures were held in a stationary position at 36°C, and medium was changed twice each week. Samples of fluids from infected cultures were pooled at weekly intervals and stored at −60°C until virus testing. All time intervals stated refer back to the day of virus inoculation.

Tests for SV-40 virus were carried out in

* Agamma Calf Serum—Hyland Laboratories, Los Angeles, Calif.