thesis occurs over a broad portion of the spectrum in cells of the blue-green algae (*6*), while developmental photoinduction is restricted to a narrow band. A phycobilin chromoprotein with an absorption maximum at 650 mμ has been separated from aqueous extracts of *Nostoc muscorum* A by ammonium sulfate fractionation. This pigment may be identical to allophycocyanin, first reported by Lemberg and Bader (*7*) and more recently shown by Haxo *et al.* (*8*) to be a constituent of cyanophycean and rhodophycean cells. The close correspondence of the absorption maxima of the isolated pigment and the action spectrum peak suggests that the blue protein acts as the photoreceptor for developmental induction.

The occurrence of a triggered, nonphotosynthetic, developmental effect of light on a photosynthetic microorganism has rarely, if ever, been reported previously. However such phenomena are well known in the photoperiodic responses of higher plants. In such systems red light is also implicated as well as a protein photoreceptor (phytochrome) (*9*). Unlike the higher plant systems, developmental photoinduction in *Nostoc muscorum* A is not reversed by far-red light. It is reversed by a broad band in the 500- to 600-mμ region of the spectrum. This fact, shown in Fig. 1, accounts for the lack of a proportional developmental response to increasing doses of white light contrasted with the proportional response to light energy from the 650-mμ region of the spectrum (*10*).

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A Mechanism of the Indole Defect in Experimental Phenylketonuria

Abstract. Rats made phenylketonuric by a diet containing high levels of either phenylalanine alone, or phenylalanine and tyrosine, show a marked reduction in total cerebral stores of serotonin. Evidence from studies both in vitro and in vivo indicates that an important mechanism of this impairment in the metabolism of serotonin is the inhibition by high levels of these amino acids of the active transport of the precursor of serotonin, 5-hydroxytryptophan, into brain.

A defect in indole metabolism in phenylketonuric patients, expressed as changes in urinary indoleacetic, indolelactic, and 5-hydroxyindoleacetic acids, as well as in blood serotonin, is now well documented (*1–3*). Auerbach and co-workers (*4*) have demonstrated similar biochemical changes, as well as a learning deficit, in rats made phenylketonuric by a diet containing 2.5 percent phenylalanine and 2.5 percent tyrosine. Recently, these alterations in indole metabolism have been shown to be reflected in lowered levels of serotonin in brain as well as in blood (*5–7*) and to be accompanied by a deficiency in maze-running ability (*6, 7*). In general, these reductions in serotonin appear to be attributable to an inhibition of the formation of serotonin. Although inhibition of both 5-hydroxytryptophan decarboxylase and tryptophan hydroxylase by phenylalanine and certain of its metabolic derivatives have been implicated as a result of experiments in vitro (*3*), no convincing evidence has yet appeared to prove that such inhibition is the primary mechanism of the indole defect in vivo.

Studies in our laboratory with slices of rat brain (*7*) have shown that an active transport mechanism exists for 5-hydroxytryptophan (5-HTP). This transport is not influenced by several potent psychopharmacologic agents, but is markedly inhibited by phenylalanine (and certain other amino acids) in the medium. The experiments described below support the thesis that this inhibition of the transport of 5-HTP into brain in vivo is an important mechanism in the production of reduced brain levels of serotonin seen in the phenylketonuric rat.

Some rats were given a single, large (1000 mg/kg) intraperitoneal injection of one of the following: D- or L-phenylalanine, D- or L-tryptophan, L-tyrosine, or L-leucine; other rats received a combination of L-phenylalanine and L-tyrosine (500 mg/kg of each). After 10 minutes, 160 μg of 5-HTP-1-C^{14} (2.5 μc per animal) was given intraperitoneally. Twenty minutes later the animals were sacrificed and determinations were made of the total cerebral content of radioactivity in ethanolic extracts of the brains, as a reflection of the amount of 5-HTP-1-C^{14} that reached the brain. These measurements were made by means of a Packard Tricarb liquid scintillation counter. In other experiments the animals received the amino acids chronically in the diet, as described below, and at the end of 2 weeks the total cerebral content of radioactivity 20 minutes after an intraperitoneal injection of 160 μg of 5-HTP-1-C^{14} was again determined. A composite of results from some of the above studies may be seen in Table 1. It is evident from these data that all the amino acids tested, *except the* D- *forms,* caused an inhibition of the uptake of 5-HTP by brain in the various procedures. These results were comparable to those obtained in our previous studies with rat brain slices (*8*). It is of interest that as much as 200 mg/kg (intraperitoneally) of sodium phenylpyruvate produced no significant inhibition.

After the ingestion of 3.5 percent phenylalanine and 3.5 percent tyrosine in the diet (*9*) for 14 days, the rats were given an intraperitoneal injection of 5-HTP (100 mg/kg) and then were sacrificed after 8 to 20 minutes. The freshly excised brains were sequentially extracted for serotonin and 5-HTP by successive extractions with alkaline and acidic butanol (*7*). The data showed that, after 8 minutes, there is a marked inhibition of the rate of appearance of 5-HTP in the brains of animals on the amino acid–supplemented diet, and this is reflected in the finding that the increase in cerebral serotonin level was only 46 percent of the control, at the end of the 20-minute time span.

At the end of 2 weeks, a group of rats on the above-described regimen of phenylalanine and tyrosine in the diet were given 160 μg of 5-HTP-1-C^{14} (2.5 μc) per animal by the intraperitoneal route, and thereafter sacrificed at various time intervals. The total radioactivity of ethanolic (80 percent) extracts of blood and brain was measured in a Packard Tricarb liquid scintillation counter. Figure 1 depicts graphically the results of a typical experiment. These data clearly show that in the phenylketonuric rat 5-HTP moves from the abdominal cavity into the

Table 1. Influence of single and repeated doses of certain amino acids on uptake of 5-HTP by rat brain in vivo. Each treatment group contained at least six rats. For quantities of amino acids and 5-HTP administered, see text. Studies involving separation of indoles by column chromatography indicated that more than 80 percent of the total radioactivity in the brain could be accounted for by unchanged 5-HTP-1-C^{14}.

Amino acid administered before 5-HTP	Brain level of 5-HTP (%)	
	Parenteral administration of amino acid	Dietary administration of amino acid
None	100	100
L-Leucine	65	70
L-Tryptophan	50	
D-Tryptophan	97	
L-Phenylalanine	42	56
D-Phenylalanine	85	
L-Tyrosine		59
L-Phenylalanine plus L-tyrosine	57	56

blood at the same rate as it does in the normal rat, but after 8 minutes there is a striking retardation in the uptake of 5-HTP by the brain of the phenylketonuric rat.

It has been demonstrated in other laboratories (*4–6*), and also by us (*7*), that animals made phenylketonuric by appropriate diets have high circulating levels of phenylalanine and tyrosine. It is reasonable to conclude, therefore, that the high blood levels of the appropriate amino acids in our experiments excluded 5-HTP from the brain. Our data strongly suggest that this exclusion is attributable to competition with 5-HTP for the normal transport mechanism in brain. In view of the demonstration of high blood levels of phenylalanine in individuals with phenylpyruvic oligophrenia, it appears reasonable to expect that a similar mechanism of interference may operate in this disease with a consequent reduction of serotonin in cerebral cells.

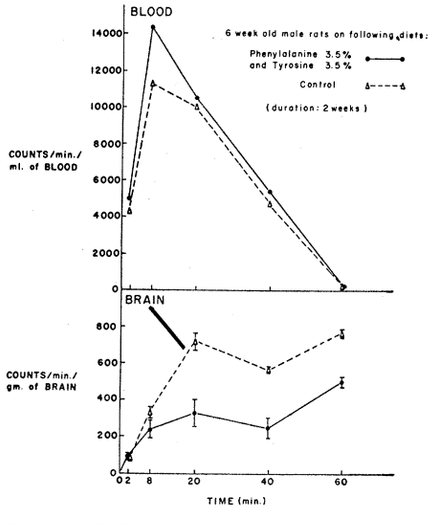

Fig. 1. Influence of chronic (2 weeks) feeding of phenylalanine and tyrosine on rate of appearance of 5-HTP-1-C^{14} in blood and brain after intraperitoneal administration of 2.5 μmole of the labeled compound.

While these experiments have not ruled out the possibility that the inhibition of tryptophan hydroxylase and of 5-HTP decarboxylase may contribute to the lowered levels of serotonin in the brain of the phenylketonuric rat, they do, by direct measurement, indicate that inhibition of the uptake of 5-HTP by the brain of such an animal is an important, if not primary, mechanism of the defect in serotonin metabolism. It is of some interest that the active transport of 5-HTP is not altered by α-methyldihydroxyphenylalanine (*8*), an inhibitor of 5-HTP decarboxylase. This would suggest that the uptake of 5-HTP by brain is independent of the activity of 5-HTP decarboxylase within the brain.

Christensen *et al.* (*10*), working with Ehrlich ascites tumor cells, first suggested that competitions may exist among amino acids for the carrier mechanism responsible for their uptake. Our data support this idea and suggest the general concept that abnormally high levels of one or more amino acids may result in a disturbance of the normal active transport of other amino acids across various anatomic barriers, such as the perivascular glial membrane, gastrointestinal mucosa, and renal tubular epithelium. Udenfriend (*11*) has recently proposed a similar concept, with respect to brain, based upon studies of the uptake of tyrosine by that organ.

Means of reversing the indole-disturbance reported above, within the framework of this general hypothesis, are now under consideration in our laboratory. Preliminary experiments (*7*) with young rats on phenylketogenic diets (described above), performing in a water maze, demonstrated that these animals were less proficient than controls, in their greater number of errors and slower performance. When blood levels of phenylalanine and tyrosine were lowered by supplementing the phenylketogenic diet with 5 percent tryptophan, presumably by competitive inhibition of the transport of phenylalanine across the intestinal mucosa, the biochemical and behavioral disturbances were reversed. The addition of 7 percent glucose to the phenylketogenic diet, while not appreciably lowering amino acid levels in the blood, showed an inconsistent and less striking but nevertheless significant improvement in both the biochemical and behavioral defects. This response to glucose supplementation is apparently through a different mechanism than that proposed for tryptophan (*12*).

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Chemical Effect of Ionizing Radiation on Cytosine

Abstract. The formation of uracil and the breakdown of the pyrimidine ring were observed when an aqueous solution of cytosine was irradiated. These two effects were investigated with the aid of radioactive tracer techniques and ultraviolet absorption studies.

In a further study of the radiation chemistry of nucleic acid constituents (*1, 2*) we have examined the chemical effect of ionizing radiation on cytosine.

In the nucleic acids, there are three pyrimidine bases—cytosine, uracil, and thymine. Uracil is found only in RNA, while thymine occurs only in DNA. Cytosine, however, is a constituent of both DNA and RNA and is the only aminopyrimidine there. In view of the observed deamination of adenine (*1*),