PHENYLPYRUVIC OLIGOPHRENIA

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Phenylpyruvic oligophrenia is an inherited disease characterized by mental deficiency and urinary excretion of phenylpyruvic acid.* Patients with this disorder are unable to carry out a particular enzymatic step in the metabolism of phenylalanine; this reaction is the conversion of phenylalanine to tyrosine:

\[
\begin{align*}
\text{NH}_2 & \quad \text{Phenylalanine} \\
\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}_2 + \frac{1}{2} \text{O}_2 & \rightarrow \text{HO-CH}_2-\text{CH}-\text{COOH} \\
& \quad \text{Tyrosine}
\end{align*}
\]

These individuals therefore appear to be human mutants, analogous to mutants such as those which have been induced in Neurospora crassa and Escherichia coli. However, in contrast to the enzymatic defect in certain mutant microorganisms, which is often recognizable in terms of a specific nutritional requirement, the metabolic block in phenylpyruvic oligophrenia is associated with a relatively complex picture.

Phenylpyruvic oligophrenia is a recessive trait; it has been estimated that the gene is carried by approximately 0.5 to 1% of the population. Although most patients with this disease exhibit markedly reduced mental capacity, a few show only moderate retardation. A high percentage of patients with phenylketonuria have fair skin, blue eyes and blond hair. About 25% of patients with phenylpyruvic oligophrenia have experienced seizures, and many of these patients have been reported to show abnormal electroencephalograms. Several necropsies have revealed evidence of abnormal myelination; on the other hand, other post-mortem studies have been described as essentially negative.

Patients with phenylpyruvic oligophrenia may excrete as much as 1 to 2 gm each of phenylpyruvic acid and phenylactic acid per day; these compounds are not usually detectable in normal urine (Fig. 1). Urinary excretion of phenylalanine, normally no more than about 30 mg/day, may be as high as 1 gm/day in phenylpyruvic oligophrenia.

* Phenylpyruvic oligophrenia (phenylketonuria) was first described by Fölling in 1934. Since this time, more than 300 cases have been reported in the literature. Reviews have appeared describing the incidence, genetic considerations, physical and mental findings, and pathologic studies.

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which is present in normal urine (about 300 mg/day), may be excreted in considerable quantities (about 1 to 3 gm/12-14).

Phenylalanine accumulates in the blood; concentrations of 10 to 60 mg/100 ml of blood plasma have been observed (normal range 1 to 3 gm2/3).

High concentrations of phenylalanine have also been observed in the spinal fluid. In contrast, relatively little phenylpyruvic acid, phenyllactic acid and phenylacetic acid have been detected in the blood of patients with phenylpyruvic oligophrenia.2/6

Studies on one newborn with phenylketonuria have been reported; the phenylalanine content of the umbilical cord blood was normal, and the concentration of phenylalanine in the serum rose to abnormal levels within a few days after birth. This infant did not excrete a detectable quantity of phenylpyruvic acid until 34 days after birth.17

In considering the biochemical phenomena associated with phenylpyruvic oligophrenia, it is desirable to review briefly the present status of knowledge concerning the intermediary metabolism of phenylalanine and tyrosine. A scheme summarizing some of this information is given in Figure 2. The available evidence (derived to a considerable extent from studies on other mammals) indicates that the major quantitative pathway of phenylalanine degradation is conversion to tyrosine. Tyrosine is subsequently converted by transamination to p-hydroxyphenylpyruvic acid, which is oxidized to homogentisic acid, which in turn is oxidized to fumaric and acetoacetic acids.

An alternative pathway of phenylalanine metabolism involves transamination to phenylpyruvic acid; further metabolism of this \( \alpha \)-keto acid yields phenyllactic acid and phenylacetylglutamine, the latter probably via the intermediate formation of phenylacetic acid. The enzymatic coupling of phenylacetic acid and glutamine (reaction 9, Fig. 2) is unique in that this reaction takes place only in human tissues (and possibly those of the chimpanzee).10-21 In the monkey, dog, rat, rabbit and other lower forms, phenylacetic acid is coupled with glycine to form phenylacetylglucose (phenylaceturic acid). It would therefore appear that the metabolic pathways of phenylalanine and of glutamate in man are unique in this respect as compared with most of the other mammals.

Information is now available concerning the enzyme system responsible for phenylacetylglutamine formation in human kidney and liver.22/23 The evidence suggests that phenylacetyl-adenylate (phenylacetyl-AMP) and phenylacetyl-coenzyme A (phenylacetyl-CoA) are intermediates in this process:

\[
\begin{align*}
(1) & \quad \text{Phenylacetate} + \text{ATP} \longrightarrow \text{Phenylacetyl-AMP} + \text{PP} \\
(2) & \quad \text{Phenylacetyl-AMP} + \text{CoA-SH} \longrightarrow \text{Phenylacetyl-CoA} + \text{AMP} \\
(3) & \quad \text{Phenylacetyl-CoA} + \text{Glutamine} \longrightarrow \text{Phenylacetylglutamine} + \text{CoA-SH}
\end{align*}
\]

The enzyme that catalyzes reaction (3) has been purified from human liver and kidney and appears to be present only in human tissues.24 As will be discussed below, the conversion of phenylalanine to phenylpyruvic acid and of the latter compound to phenyllactate and phenylacetylglutamine, normally of minor quantitative significance, become the major degradative pathways of phenylalanine metabolism in phenylpyruvic oligophrenia.1 The reduction of phenylpyruvate to phenyllactate may be catalyzed by a preparation of liver obtained by biopsy from a patient with phenylketonuria was found to form phenylacetylglutamine at approximately five times the rate observed with liver preparations obtained from patients without phenylketonuria.25 It would appear that this enzyme system is more active in individuals with phenylketonuria and may possibly be adaptive in nature; however, final conclusions must await studies on additional samples of phenylketonuric liver.
by lactic dehydrogenase, an enzyme known to exhibit a broad substrate specificity.\textsuperscript{26}

That the conversion of phenylalanine to tyrosine (reaction 1, Fig. 2) was impaired in phenylpyruvic oligophrenia was suggested by the observation that although administration of phenylalanine to normal subjects resulted in an increase in the Millon-reactive substances of the blood, phenylalanine administration did not increase the level of Millon-reactive compounds in the blood of patients with phenylpyruvic oligophrenia.\textsuperscript{27} Unequivocal proof of a deficiency in the conversion of phenylalanine to tyrosine was provided by experiments in which radioactive phenylalanine was administered to patients with phenylketonuria and the specific activity of the phenylalanine and tyrosine of the plasma proteins subsequently determined. In contrast to results in individuals without phenylketonuria, very little radioactivity was found

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1}
\caption{Fig. 1.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{Fig. 2. Scheme of the metabolism of phenylalanine and tyrosine (adapted from Meister\textsuperscript{24}).}
\end{figure}
in the isolated tyrosine.\textsuperscript{28} Since a small amount of radioactive tyrosine was formed, it is possible that the block may not be complete, or that some conversion of phenylalanine to tyrosine is carried out by the bacterial flora.

Study of liver specimens obtained from patients with phenylketonuria at necropsy or operation failed to reveal evidence of any enzymatic activity capable of converting phenylalanine to tyrosine, whereas liver specimens obtained from individuals with other conditions catalyzed the conversion of phenylalanine to tyrosine.\textsuperscript{29,30} The mechanism of the enzymatic conversion of phenylalanine to tyrosine is not as yet completely understood. However, it has been found that the reaction, which apparently takes place exclusively in the liver, requires the participation of diphosphopyridine nucleotide or triphosphopyridine nucleotide and oxygen.\textsuperscript{31-33} The enzyme system can be separated into two fractions:\textsuperscript{32} One of these (Fraction I) is relatively labile and is found solely in the liver, while the other (Fraction II) is more stable, and is found in certain other tissues as well. A lack of either Fraction I or Fraction II (or of both fractions) could be responsible for the marked decrease in the conversion of phenylalanine to tyrosine in phenylpyruvic oligophrenia. If patients with phenylketonuria were unable to synthesize Fraction II, then one might expect abnormal metabolic function in a number of tissues (perhaps including the brain). On the other hand, a deficiency of Fraction I would represent a specific hepatic defect.

In an attempt to shed light on this problem, a study of the conversion of phenylalanine to tyrosine in phenylpyruvic oligophrenia was undertaken in the author's laboratory.\textsuperscript{30} An enzyme preparation obtained from liver obtained by biopsy from a patient with phenylpyruvic oligophrenia did not catalyze detectable conversion of phenylalanine to tyrosine. However, when a purified preparation of Fraction I obtained by fractionation of rat liver was added to the phenylketonuric-liver preparation, a very considerable conversion of phenylalanine to tyrosine was observed (Fig. 3). An analogous experiment in which Fraction II (also prepared from rat liver) was added to the phenylketonuric-liver preparation was also carried out; in this case only a small conversion of phenylalanine to tyrosine was observed. This relatively small effect was consistent with the fact that neither of the purified fractions was completely free of the other. The striking activation of the conversion of phenylalanine to tyrosine produced by addition of rat Fraction I to the phenylketonuric-liver preparation represents strong evidence for the absence of Fraction I in the phenylketonuric liver.

In these experiments, it was not feasible to separate the small quantity of liver removed at operation into fractions corresponding to Fractions I and II. In independent studies by Mitoma et al.\textsuperscript{34} liver specimens obtained from patients with phenylketonuria at necropsy were fractionated in this manner. The presence of activity corresponding to Fraction II was demonstrated in the necropsy liver specimens. Unfortunately, Fraction I could not be demonstrated in liver obtained from individuals either with or without phenylketonuria; presumably, Fraction I, was inactivated soon after death.

Nevertheless, demonstration of Fra-
tion II activity in the liver of patients with phenylketonuria is consistent with the conclusion that the metabolic defect in phenylpyruvic oligophrenia is associated with a deficiency of Fraction I. The defect therefore appears to be primarily one of the specific hepatic function rather than one which might be expected to manifest itself in a number of tissues. It should be emphasized that studies thus far have dealt only with enzyme activity. The available data do not answer the question as to whether the enzyme is absent, or whether it is present in an inactive state.

It is not immediately clear as to how a deficiency in the conversion of phenylalanine to tyrosine may be related to the extraordinary degree of mental retardation associated with this disease. It does not seem likely that individuals with phenylketonuria suffer from a marked deficiency of tyrosine since this amino acid is probably available in sufficient quantities in the diet. Furthermore, tyrosine supplementation of the diet does not ameliorate the condition. However, tyrosine must be considered a dietary essential amino acid for patients with phenylketonuria, although this has not yet been rigorously demonstrated in experiments of the type performed by Rose.

It is conceivable, of course, that the cerebral lesion and the hepatic abnormality are separate phenomena not causally related. However, there is evidence consistent with the belief that these defects are related. Studies on microorganisms have provided much support for the "one gene-one enzyme" concept; the development of phenomena secondary to a block is not uncommon in mutant microorganisms.

A relationship between the enzymatic defect in the liver and the cerebral lesion may become understandable in terms of the consequences of the accumulation of phenylalanine. That such accumulation occurs is evident from the high concentrations of phenylalanine in the blood, and the increased urinary excretion of this amino acid. An obvious consequence of phenylalanine accumulation is increased formation of phenylpyruvic acid. An increased concentration of phenylalanine would be expected to drive reaction 2 (Fig. 2) in the direction of phenylpyruvic acid formation, and more of this a-keto acid would therefore be available for conversion to phenylactic acid, phenyllactic acid and phenylacetlylglutamine. The conversion of tyrosine to p-hydroxyphenylpyruvic acid (reaction 3, Fig. 2) takes place by transamination, and it is probable that the conversion of phenylalanine to phenylpyruvate proceeds by a similar mechanism.

Since these transamination reactions are reversible, it would be expected that less phenylpyruvic acid might be formed in the presence of increased concentrations of amino donors. Thus, a reduction in the excretion of phenylpyruvic acid and phenylactic acid by patients with phenylketonuria was observed following relatively large oral doses of certain amino acids. For example, a significant reduction of phenylpyruvic acid excretion was observed in an 18-kilogram girl with phenylketonuria 1 hour after administration of 136 millimoles of L-glutamine; a minimum excretion of about 40% of the control value was reached 4 hours after administration of glutamine, and the excretion of keto acid returned to the control level after 6 hours. A considerable increase in the excretion of a-ketoglutаратate was observed during the period of reduced phenylketonuria.

Similar findings were observed in two patients with phenylketonuria after administration of L-glutamate and L-asparagine; however, administration of sodium succinate, glycine or D-glucose had no effect on phenylpyruvic acid excretion. It is of interest that the excretion of phenylacetlylglutamine was unchanged by the administration of glutamine; the decrease in phenylpyruvic acid formation may not have

* It is of interest to note that alcaptonuria, a condition associated with a major block in tyrosine metabolism (deficiency of homogentic acid oxidation, reaction 5, Fig. 2), is not accompanied by serious systemic or cerebral complications.
been great enough to affect the formation of phenylacetylglutamine.

Since administration of glutamine did not increase phenylacetylglutamine excretion, it appears unlikely that there is a deficiency of glutamine for the coupling reaction, and there is no reason to believe that the excretion of phenylacetylglutamine by patients with phenylketonuria represents a serious loss of glutamine from the body.

The results of these studies on the effects of administration of amino acids in phenylpyruvic oligophrenia are consistent with the belief that the conversion of phenylalanine to phenylpyruvic acid is a transamination reaction. The possible therapeutic effectiveness of amino acid administration requires further study and consideration. Formation of products of phenylalanine metabolism (e.g., phenylpyruvic acid and phenylactic acid) may be reduced by such amino acid administration; however, a greater accumulation of phenylalanine would also be expected to occur.

Although it is plausible that the mental defect in phenylpyruvic oligophrenia is a consequence of high concentrations of phenylalanine or of metabolites of this amino acid, the mechanism of such an effect is at present obscure. Evidence in favor of this hypothesis arises from studies in which patients with phenylketonuria have been administered diets containing very low levels of phenylalanine. Improvement has been reported in some patients. The effects of dietary phenylalanine restriction on the urinary excretion of phenylpyruvic acid and on the concentration of phenylalanine in the blood are dramatic; the excretion of phenylpyruvic acid may decrease to virtually zero and the phenylalanine in the blood may approach normal concentrations. It has also been reported that patients who previously experienced seizures have exhibited less tendency to have such attacks when given a low phenylalanine diet. In some cases, the electroencephalographic findings appeared to become more normal.

The improvement observed in patients receiving the restricted phenylalanine diet is consistent with the hypothesis that high blood and tissue concentrations of phenylalanine or products of phenylalanine metabolism may be responsible for the cerebral defect. Thus, high concentrations of phenylalanine, phenylpyruvic acid, phenylactic acid or phenylacetic acid, for example, might produce cerebral damage or prevent normal cerebral development at a crucial stage.

The possible toxicity of phenylacetic acid has been considered by several investigators. Other compounds have been reported to be excreted in greater than normal quantities in phenylketonuria; these include o-hydroxyphenylacetic acid, p-hydroxyphenylacetic acid, p-hydroxyphenylactic acid, indoleacetic acid and indolelactic acid. The presence of relatively large amounts of these products may represent abnormalities in the metabolism of tyrosine and tryptophan, due to inhibition by high concentrations of phenylalanine or phenylpyruvic acid, or may possibly be caused by an adaptive increase of the enzyme systems concerned with the conversion of phenylalanine to phenylpyruvate, phenylactate and phenylacetylglutamine. An increase in transaminase activity, for example, might result in a greater conversion of tryptophan and tyrosine to their respective a-keto acid analogues, which in turn, could be reduced or decarboxylated (Fig. 3).

The presence of o-hydroxyphenylacetic acid in the urine of patients with phenylpyruvic oligophrenia is somewhat surprising since o-tyrosine is not known to be a natural metabolite. o-Hydroxyphenylacetic acid has been shown by isotopic studies to...
arise from phenylalanine, the mechanism of its formation is not yet known. It is of considerable interest that the excretion of these compounds has been reported to decrease when patients with phenylketonuria follow a phenylalanine-restricted regimen. It is possible that high concentrations of phenylalanine in blood and tissue result in an amino-acid imbalance which interferes with the metabolism of other amino acids (especially tyrosine) and perhaps also with protein synthesis. High concentrations of phenylalanine have been found to inhibit mushroom tyrosinase competitively, and similar findings have been made with mammalian tyrosinase; these observations suggest a mechanism for the toxicity of phenylalanine. It may be observed, in this connection, that some patients with phenylketonuria have shown an increase in pigmentation of skin and hair after receiving a low-phenylalanine diet for several months. There are now many examples of experimentally induced amino-acid imbalance, and it is evident that a naturally occurring amino acid, if present in sufficient concentration, may act as an amino-acid antagonist. Both phenylalanine and phenylpyruvic acid have recently been found to interfere with metabolism of tyrosine in in-vitro studies. High concentrations of phenylalanine might also affect formation of thyroid hormone or of epinephrine and norepinephrine. It has recently been shown that certain amino-acid antagonists are incorporated into proteins in place of their naturally occurring analogues. Phenylalanine may inhibit synthesis of certain proteins (perhaps as a tyrosine or tryptophan antagonist), or, in the presence of high concentrations of phenylalanine, abnormal proteins containing large amounts of this amino acid may be formed. Although studies on the phenylalanine content of proteins from patients with phenylketonuria have been carried out, further work would be desirable. It is of interest that serum obtained from some patients with phenylketonuria has been reported to contain abnormal \( \beta \)-globulins; these disappear when the patients are placed on restricted phenylalanine diets.

Although several plausible mechanisms for the toxic effect of phenylalanine may be formulated (Fig. 4), a complete biochemical explanation of the cerebral defect is still lacking. The current procedure of...
giving patients with phenylketonuria a restricted dietary intake of phenylalanine is a rational therapy and has apparently been at least partly successful.

Greater success may attend studies on very young infants; if this is the case, the importance of early diagnosis becomes obvious.\(^*\) It remains to be demonstrated that normal cerebral development can be achieved by instituting a restricted phenylalanine diet from birth in affected individuals. Until this is accomplished, the possibility that abnormalities exist other than those directly related to phenylalanine accumulation cannot be conclusively excluded. It must also be noted that it is not yet certain that the mental defect is irreversible in older patients.

Although some improvement has been observed with the low phenylalanine regimen, other approaches should probably not be abandoned. It is possible that ways can be found to promote the detoxification and the excretion of phenylalanine and its metabolites. The possibility of producing a bacterial flora in the intestine capable of converting phenylalanine to tyrosine, although perhaps difficult to achieve, deserves at least brief mention. However, it will be of utmost importance to pursue actively research on the enzyme system that catalyzes the para-hydroxylation of phenylalanine, and to determine ultimately the exact nature of the genetic and molecular defects in phenylpyruvic oligophrenia. It will be necessary to learn whether the enzyme is absent or whether it is present in an inactive form. Such information will be essential for the development of therapy based on enzyme replacement or enzyme activation. This type of information, as well

\(^*\) It has recently been reported that parents of patients with phenylketonuria exhibit prolonged high concentrations of phenylalanine in the blood after oral test doses of this amino acid.\(^*\) These individuals exhibit none of the signs or symptoms of phenylpyruvic oligophrenia. This important finding may lead to the development of a procedure for the detection of heterozygous individuals, and it provides additional evidence for the hypothesis that the primary lesion in phenylketonuria is a disturbance of the conversion of phenylalanine to tyrosine.\(^*\)
as data on the pathogenesis of the cerebral lesion, will be of importance not only in terms of this disorder, but may well be significant for the understanding of other disease phenomena.

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REFERENCES


CONGENITAL DEFECTS IN ADRENAL STEROID SYNTHESIS

By Alfred M. Bongiovanni, M.D.

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SCRUTINY of the adrenocortical steroids excreted in the urine of patients with the adrenogenital syndrome (due to congenital adrenal hyperplasia) has afforded an opportunity to localize the biochemical defects attributable to an inborn error of metabolism and has elucidated the normal pathway for biosynthesis in the adrenal gland in man.1-3 The studies of Hechter on the biogenesis of hydrocortisone in the beef adrenal has indicated a stepwise oxidation of progesterone toward the fulfillment of the requirements for the final product, namely hydrocortisone. Hydrocortisone is indeed a "final product" in the economy of the pituitary adrenal axis: When its rate of secretion is low, larger amounts of adrenocorticotropic hormone (ACTH) are released in an attempt to stimulate the adrenal cortex. If the target gland cannot properly synthesize hydrocortisone, the pituitary continues to stimulate the gland, which produces large quantities of "abnormal" intermediary metabolites. Certain of these latter substances are androgenic and account for the clinical manifestations.

An oxygen function must be introduced into the progesterone molecule at carbons 17, 21 and 11 in order for the adrenal cortex to manufacture hydrocortisone. The oxygen is introduced at each position, presumably under the control of separate enzymes, tentatively termed "hydroxylases" and specified by the position they affect. If one or more of these enzymes is lacking, hydrocortisone is not produced, or is synthesized in limited quantities. Depending upon the particular enzymatic defect, the usual metabolites measured in the blood and urine vary, as does the type of disease encountered.

In all forms of the adrenogenital syndrome, virilization is present. The disease is initiated in utero so that female infants...
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