# Phenylketonuria

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THE observation by Fölling[1] that certain mentally defective subjects excreted phenylpyruvic acid in the urine represents the first demonstration of a specific chemical defect in a mental disorder. Although there have since appeared other substantiated examples of such chemical correlations with mental disease, phenylketonuria remains the best studied example of them all. Since Fölling's original observation, we have learned a great deal about the genetic nature of this disorder, the readily observable enzymatic lesion and even about effective therapy. However, the most interesting problem of all, the one concerning the reasons for the mental derangement in this disease, is still unsolved. Solution of this problem may well pave the way for future advances in the chemical pathology of the central nervous system.

Phenylketonuria is transmitted as a recessive characteristic from a single autosomal gene. The heterozygous parents, who apparently are normal, give rise to a certain percentage of homozygous phenylketonuric offspring. The incidence of phenylketonuria in the general population has been estimated at between one in 25,000 to one in 50,000.[2] It may be estimated, therefore, that one person in 100 may be a heterozygous carrier of this disease.

Advances in methodology have made it possible to detect and measure many chemical changes associated with this disorder, and we know that, in addition to the excretion of phenylpyruvic acid in the urine, there is a marked elevation in phenylalanine blood levels. (Normal subjects, 1 mg. per 100 ml.; untreated subjects with phenylketonuria, 20 to 60 mg. per 100 ml.). The large amounts of phenylalanine represent an inability to metabolize this amino acid, and it is now recognized that this is due to a block in the major route of phenylalanine metabolism, hydroxylation to tyrosine.

Phenylalanine hydroxylation requires two protein catalysts, one is present only in liver and the other is more generally distributed. Additional requirements are reduced pyridine nucleotide, and a pteridine-like cofactor (Fig. 1). Studies in several laboratories have pinpointed the lesion as being a marked deficiency in the liver enzyme, the other enzyme and the cofactors being present in normal amounts in the liver of phenylketonuric subjects. If we accept this one enzyme deficiency as the only genetically linked disturbance, then it must be concluded that phenylketonuria is primarily a hepatic disorder and that the mental retardation is a secondary effect. I would like to discuss some possible mechanisms of such a secondary effect.

## PHENYLALANINE METABOLISM

In the absence of phenylalanine hydroxylase, phenylalanine metabolism is markedly diminished. Large amounts of the amino acid persist in the tissues for sufficiently long periods of time so as to yield quantities of products which are normally formed and excreted only in trace amounts (Fig. 2). Thus, transamination of phenylpyruvic acid becomes a major metabolic pathway. O-Hydroxyphenylacetic acid, which is normally excreted in microgram amounts, is excreted to the extent of several hundred milligrams per day. Many other acidic and phenolic products have been found in the urine and blood of phenylketonuric subjects but none possess pharmacologic ac-

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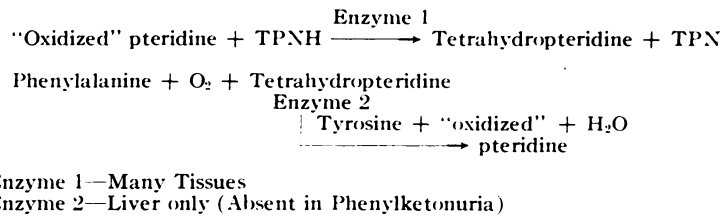


FIG. 1. Enzyme and cofactor requirements for hydroxylation of phenylalanine.

tivity which can be associated with a centrally active agent. Recently Jepson et al.[3] reported that traces of phenylethylamine are normally excreted in the urine. Following administration of monoamine oxidase inhibitors, phenylketonuric subjects excreted more than 3 mg. of phenylethylamine per day, whereas normal subjects excreted less than 0.05 mg. per day. Since even with monoamine oxidase inhibitors intravenously administered phenylethylamine was poorly recovered in the urine, it was estimated that in phenylketonuric subjects as much as 50 mg. of this amine may be synthesized per day.

Unlike the phenolic acids, phenylethylamine is a pharmacologically active substance which possesses properties like amphetamine, although of weaker activity. It is now known that phenylethylamine is formed by decarboxylation of phenylalanine in mammalian tissues. It is of further interest that the enzyme, aromatic L amino acid decarboxylase, is present in many organs. It is also present in brain in which it is localized in the brain stem areas.[4] Furthermore, the affinity of phenylalanine for the decarboxylase is low so that saturating levels are not reached. In the presence of increased amounts of phenylalanine, the rate of decarboxylation is increased. Thus, in the brains of phenylketonuric subjects it is quite probable that phenylethylamine production takes place at a rate twenty to sixty times normal, since it is fairly certain that brain levels are comparable to blood and spinal fluid levels. Phenylethylamine may very well be the "*neurotoxic*" substance suggested by Armstrong[5] and by others. It may be that the presence of such a pharmacologically active substance during postnatal development of the brain may either interfere with normal learning (imprinting), or it may have more direct effects on brain chemistry. Such effects could very well be irreversible.

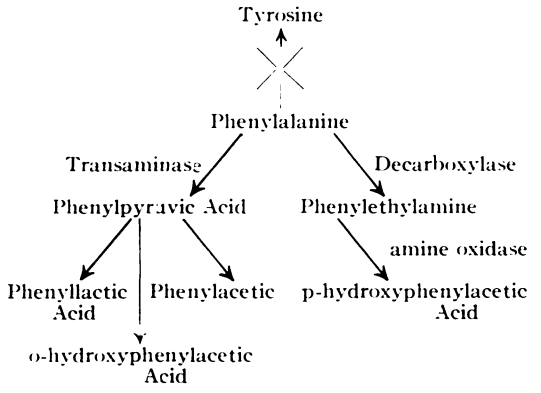


FIG. 2. Alternate pathways for phenylalanine metabolism.

Another suggested mechanism for the central defect comes from the reports of Pare, Sandler and Stacey[6] on 5-hydroxyindole metabolism. They have shown that both plasma serotonin and urinary 5-hydroxylindoleacetic acid levels are considerably lower in phenylketonuric subjects as compared to other mentally defective subjects. Sandler and Stacey discuss the possibilities of intereference with tryptophan hydroxylation or 5-hydroxytryptophan decarboxylation by phenylalanine or its metabolites. An alternate explanation of their findings is interference with transport mechanisms for serotonin and its metabolites in kidney and blood platelets. Of course, we still know little about the function of serotonin in brain. The large amounts of phenylalanine and its metabolites appear to influence many metabolic pathways including those relating to tryptophan, catecholamines and γ-aminobutyric acid. Any one of these may be of significance in the brain disturbance.

TABLE I
Effects of Amino Acid Antagonists on Increase in Brain Levels of L-Tyrosine After its Administration

| Amino Acid | Increase in Brain Level (μg./gm.) | | |
|---|---|---|---|
| | 30 Minutes | 60 Minutes | 120 Minutes |
| Control | 44 ± 2 | 61 ± 2 | 62 ± 3 |
| L-Tryptophan | 9, 12, 5, 8 | 24, 26, 15, 23 | 60, 33 |
| β-Fluorophenyl-DL-alanine | 11, 4 | 25, 13 | 28 |
| L-Leucine | 2, 1 | 7, 7 | 21, 30 |

TABLE II
Phenylalanine Hydroxylase Activity in Liver of Premature Human Infant

| Preparation | Additions | Tyrosine Formed (μM) |
|---|---|---|
| Soluble fraction | ... | 0.0 |
| Soluble fraction | Rat liver Enzyme 2 | 0.73 |
| Soluble fraction | Rat liver Enzyme 1 | 0.0 |
| ... | Enzyme 1 + 2 | 0.98 |

NOTE: Enzyme 2 = hydroxylase. Enzyme 1 = pteridine reductase.

That phenylalanine, itself, can interfere with other enzymes was definitely shown by the studies of Dancis et al.[7] on the enzyme tyrosinase. They showed that this enzyme system, which is involved in melanin formation, is markedly inhibited by concentrations of phenylalanine which are found in tissues of phenylketonuric subjects. They concluded that the blond hair and light coloring were the result of significant interference with pigment formation by the high levels of phenylalanine in the tissues.

Another type of interference which could specifically influence brain nutrition is one with aromatic amino acid transport. It has been shown by Chirigos et al.[8] that the uptake of tyrosine by brain, *in vivo*, depends on a catalytic mechanism. The following evidence is given (1) uptake is rapid; (2) the uptake is stereospecific; L-tyrosine is taken up much more rapidly and to a much greater extent than the D-isomer; (3) compounds structurally related to tyrosine, with similar solubility charactertisics, are not taken up to an appreciable extent; and (4) uptake is markedly diminished in the presence of certain other amino acids, L-tryptophan and fluorophenylalamine (Table I), indicating competition for the uptake mechanism. Phenylalanine could not be studied as a competitor since in normal animals it is rapidly converted to tyrosine. Neither structural specificity nor competitive inhibition by other aromatic amino acids were observed in tyrosine uptake by other tissues such as muscle *in vivo* or in *in vitro* and even brain slices do not possess the same characteristics as the *in vivo* organ.

It would appear that the catalyzed uptake of L-tyrosine (and other normal amino acids) by brain occurs at the same site as the so-called "blood-brain barrier." The presence of a catalytic mechanism for uptake of charged water-soluble nutrients into brain differentiates it from other organs and indicates that brain uptake should be susceptible to competitive inhibitors and other factors which can influence catalytic mechanisms. For this reason large amounts of one aromatic amino acid can competitively inhibit the uptake of another into the brain. Such a mechanism could explain a central action of the huge amounts of circulating phenylalanine in phenylketonuric subjects. A diminution in uptake of essential amino acids, would, of course, have many consequences.

## NUTRITIONAL ASPECTS

Although the reason for the central defect is not certain, it is now possible to remove all chemical manifestations of this disorder by lowering the dietary intake of phenylalanine sufficiently. If such dietary restriction is imposed from birth even the central aspects of this disorder can be averted.[9] Studies of Kenney and Kretchmer[10] indicate that there is no distinction between normal and phenylketonuric subjects at the fetal stage, neither containing the enzyme, phenylalanine hydroxylase (Table II). No damage should therefore occur *in utero*.

Following birth, plasma levels of phenylalanine do not rise until protein feeding is instituted. Commercially prepared low phenylalanine diets started at, or shortly after, birth appear to have a definite effect in preventing mental deficiency. However, for proper use of the diet early diagnosis is required. In Cincinnatti newborn children are sent home with filter paper patches inserted in the diapers. The patches are sent back to the laboratory for phenylpyruvic acid testing. Newborn children in families with a history of phenylketonuria should have phenylalanine blood levels assayed at frequent intervals until it can be ascertained whether they are normal or not. If high levels are found, the low phenylalanine diet should be instituted.

Detection of the heterozygous carriers of phenylketonuria appears to be a distinct possibility. Hsia et al.[11] and Knox and Messinger[12] have reported that in parents and normal siblings of phenylketonuric subjects an orally administered dose of L-phenylalanine cannot be metabolized as rapidly as in normal subjects. An oral phenylalanine tolerance test appears to distinguish heterozygotes from noncarriers. The ratio of phenylalanine to tyrosine in blood[13] and the amount of orthohydroxyphenylacetic acid in urine[14] following the oral administration of phenylalanine also appear to distinguish the heterozygote group. However, although these tests appear to be able to distinguish one group from another, they are not yet able to detect the phenylketonuric trait in a given subject because of the considerable overlap between the normal and heterozygous groups. Perhaps with more refined technics detection in given subjects may become possible.

One of the factors which has limited experimentation in this field has been the lack of an experimental animal. However, it now appears that Waisman and associates[15] have been able to produce all the chemical manifestations of phenylketonuria in monkeys by feeding large amounts of phenylalanine in the diet from birth. Psychologic testing indicates that these monkeys grow up mentally retarded. Such a test animal should prove most important in corroborating our present concepts of this disorder and in exploring the mechanism of the central defect.

It is of interest that a comparable disorder involving the leucines and valine has been discovered.[16] The term "maple-syrup" urine disease has been used because of the odor of some of the excretory products. As in phenylketonuria, the amino acid levels are markedly elevated, keto acids are excreted in the urine and central disturbance is marked. Genetically, the two are also similar. It will be of interest to compare these two disorders in greater detail as more cases become available.

## SUMMARY

It is apparent that we already know more about phenylketonuria from the genetic, biochemical and therapeutic standpoint than we do about other comparable disorders. However, recent advances indicate that we may also be able to investigate the causes of the central disturbance.

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