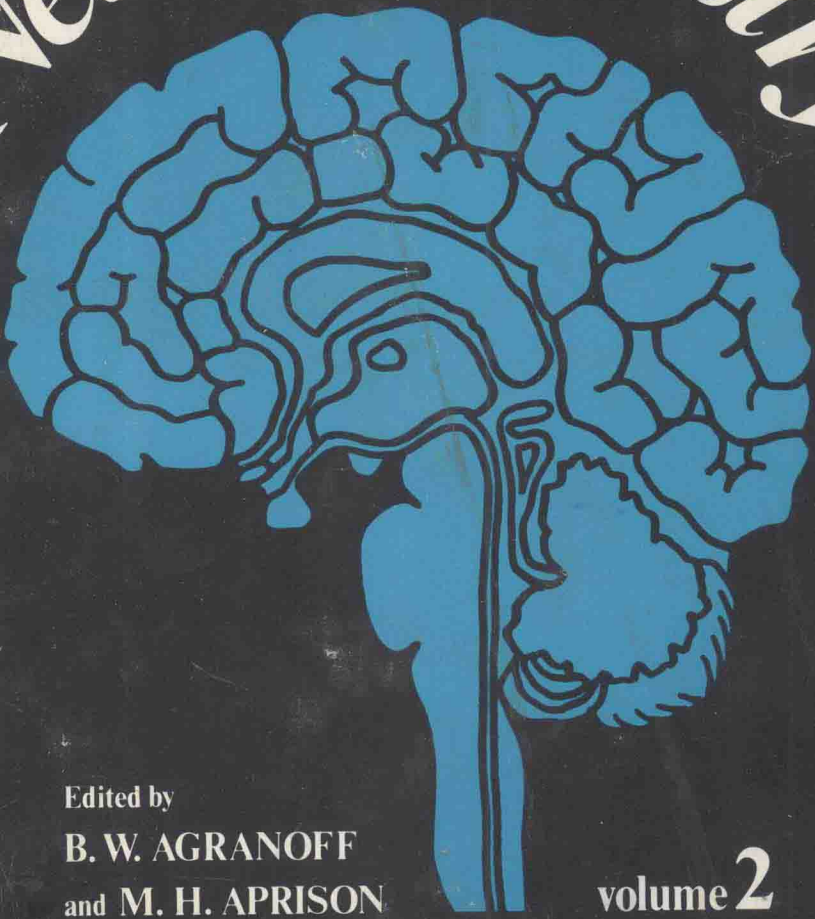
Advances in Neurochemistry



Edited by
B. W. AGRANOFF
and M. H. APRISON

volume 2

Advances in
Neurochemistry

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B. W. Agranoff

Mental Health Research Institute and
Department of Biological Chemistry
University of Michigan
Ann Arbor, Michigan

and

M. H. Aprison

Institute of Psychiatric Research and
Department of Psychiatry and Biochemistry
Indiana University School of Medicine
Indianapolis, Indiana

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CONTRIBUTORS

MANFRED L. KARNOVSKY • *Departments of Biological Chemistry and Psychiatry, Harvard Medical School, Boston, Massachusetts*

SEYMOUR KAUFMAN • *Laboratory of Neurochemistry, National Institute of Mental Health, Bethesda, Maryland*

DANIEL E. KOSHLAND, JR. • *Department of Biochemistry, University of California, Berkeley, California*

MASANORI OTSUKA • *Department of Pharmacology, Faculty of Medicine, Tokyo Medical and Dental University, Tokyo, Japan*

PETER REICH • *Departments of Biological Chemistry and Psychiatry, Harvard Medical School, Boston, Massachusetts*

T. L. SOURKES • *Laboratory of Neurochemistry, Department of Psychiatry, McGill University, Montreal, Quebec, Canada*

S. N. YOUNG • *Laboratory of Neurochemistry, Department of Psychiatry, McGill University, Montreal, Quebec, Canada*

PREFACE

In the Preface to Volume 1, we stated:

> This series recognizes that investigators who have entered neurochemistry from the biochemical tradition have a rather specialized view of the brain. Too often, interdisciplinary offerings are initially attractive but turn out to recite basic biochemical considerations. We have come to believe that there are now sufficiently large numbers of neurochemists to support a specialized venture such as the present one. We have begun with consideration of traditional areas of neurochemistry which show considerable scientific activity. We hope they will serve the neurochemist both for general reading and for specialized information. The reader will also have the opportunity to reflect on the unbridled speculation that results from the disinhibiting effects on the author who has been invited to write a chapter.
>
> We plan occasionally also to offer reviews of areas not completely in the domain of neurochemistry which we nevertheless feel to be sufficiently timely to be called to the attention of all who use chemical principles and tools in an effort to better understand the brain.

The contributions to the present volume pursue these goals. We believe the series has set high standards and has continued to uphold them. In accordance with the principle stated in the last paragraph of the Preface Volume 1, we include in this volume Koshland's "Sensory Response in Bacteria" (Chapter 5). We draw attention not so much to the fact that a bacterium has a "sense organ," but to its use of a temporal mechanism for chemoreception which may, like other bacterial mechanisms, have its counterpart in eukaryotes and in particular, in neurons.

B. W. Agranoff
M. H. Aprison

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SENSORY RESPONSE IN BACTERIA

DANIEL E. KOSHLAND, JR.

CHAPTER 1

PHENYLKETONURIA: Biochemical Mechanisms

SEYMOUR KAUFMAN

Laboratory of Neurochemistry
National Institute of Mental Health
Bethesda, Maryland

1. INTRODUCTION

As implied by the title, this review of phenylketonuria (PKU) will be neither comprehensive nor encyclopedic. Rather, it will be limited to those aspects of the disease where sufficient biochemical knowledge is available to support meaningful discussion—admittedly, often speculative—about underlying mechanisms. Recent comprehensive reviews are available (Hsia, 1970; Knox, 1972).

To those interested in the normal development and functioning of the brain, PKU—especially its pathophysiology—has held the promise of providing unique insights. The reason for this hope can probably be traced to the remarkable specificity of tissue damage that is characteristic of the disease. Thus, while mental retardation is a consequence of many untreated inborn errors of metabolism, with most of them the developing brain is but one of the organs that is adversely affected by the disease. In galactosemia, for example, mental retardation is one of the characteristic symptoms, but there is also evidence of damage to the liver, kidneys, eyes, etc. By contrast, in PKU, the damage to the brain is quite specific.

This disease also provides us with one of nature's invaluable paradigms for studying and, ultimately, understanding aspects of the dynamic

metabolic interaction between different organs; for although the brain appears to be uniquely damaged in PKU, that organ does not even possess the enzyme system that is affected in the disease—the phenylalanine hydroxylating system, which is located exclusively in the liver and kidneys. The damage to the brain in PKU, therefore, is clearly secondary to the primary defect in these distant organs.

2. HISTORY OF THE DISEASE AND ITS GENERAL CHARACTERISTICS

Surprisingly, the first biochemically signficant observation on PKU was made not by a scientist but by the mother of two mentally retarded children. When, in 1934, she brought the children to A. Fölling, a young Norwegian biochemist and physician, she mentioned to him that since early infancy, a peculiar "mousy" odor had clung to the children. Apparently suspecting that the odor might be due to the urinary excretion of acetoacetate, Fölling added ferric chloride to samples of the urine. Instead of turning the red-brown color that is characteristic of acetoacetate, the urine turned green. In a period of less than 2 months, Fölling isolated from the urine the compound responsible for the green color and identified it as phenylpyruvate (Fölling, 1934*a*) (Later it was shown that the compound responsible for the mousy odor was phenylacetate). Within a short time, Fölling discovered eight more mentally retarded patients who also excreted phenylpyruvate. In 1938, Fölling made his second important observation on the biochemical characteristics of the new disease when he reported that these patients had elevated levels of phenylalanine in their blood and urine (Fölling, 1938). He correctly postulated that the new disease was caused by an inherited defect in phenylalanine metabolism (Fölling, 1934*a*), calling it *imbecilitas phenylpyruvica*. Subsequently, it was referred to as *phenylpyruvic amentia, phenylpyruvic oligophrenia,* Fölling's disease, and phenylketonuria. The last name, introduced by Penrose and Quastel (1937), appears to have been generally adopted.

Individuals with PKU are relatively healthy in all aspects but one—they are mentally retarded. The vast majority are idiots, i.e., their I.Q.s are less than 50 (Jervis, 1954; Paine, 1957; Hsia *et al.*, 1958; Partington, 1962). They also tend to show a deficiency in pigmentation and have blond hair and blue eyes (Paine, 1957). About one-third of the patients have some degree of eczema or other skin conditions (Knox, 1972).

In addition to mental retardation, the patients show other signs of central nervous system (CNS) pathology. Most have abnormal EEG patterns, and about one-fourth have a history of convulsive seizures, usually

starting between 6 and 18 months of age. Other characteristic neurological manifestations of the disease are increased muscle tone, hyperactive tendon reflexes, tremors, and adventitious hyperkinesis (Knox, 1972). When it is not masked by severe mental retardation, disturbed behavior appears to be another salient feature of the disease. Screaming, sudden outbursts of violent activity, and increased irritability, are common. A significant number of the patients show behavior that has been interpreted as psychotic (see Cowie, 1971, for review).

Of all the symptoms of classical PKU, it is primarily the mental retardation, presumably a reflection of structural damage to the brain, that quickly becomes irreversible unless therapy (restriction of phenylalanine intake) is instituted early. Almost all the other symptoms can still be reversed by the dietary therapy long after the brain damage becomes permanent. As will be discussed, the etiology of these two kinds of symptoms (the reversible and the irreversible) are probably different.

Considering the overt signs of CNS involvement in PKU, there was a surprisingly long lag period before any pathological correlates of the disease in the CNS were established. It is now generally agreed that there is a defect in myelinization in the brains of PKU patients, which is accompanied by myelin breakdown products (Poser and Van Bogaert, 1959; Crome and Pare, 1960; Malamud, 1966; Menkes, 1968). Possible biochemical mechanisms for this defect will be discussed later.

Since effective treatment of the disease depends on early diagnosis (as will be shown), it is fortunate that there are biochemical changes that are manifested many months before the first signs of mental retardation or neurological deterioration. Nevertheless, not until it was realized that despite a lack of a functional phenylalanine hydroxylase system, the newborn PKU infant is not clinically abnormal [although it is somewhat underweight (Saugstad, 1972)], and all the urinary and blood biochemical abnormalities that characterize the disease develop postnatally, was accurate early diagnosis of PKU possible. An additional complication that compounded the first attempts at early diagnosis was ignorance of the fact that not all the biochemical abnormalities develop simultaneously.

Thus, although the elevation in blood phenylalanine in PKU infants is the earliest chemical change, and is therefore used as the basis for the diagnosis of the disease in the newborn infant, even this alteration is minimal at birth. This developmental characteristic of the disease, so critical for its reliable diagnosis in the newborn infant, was first reported by Armstrong and Binkley (1956). They showed that the concentration of phenylalanine in the blood of a PKU baby was normal at birth and that the level rose steadily thereafter, reaching a peak at 24 days. More recent studies have confirmed this finding and have shown that the maximum phenylalanine levels are reached, on the average, by the 6th or 7th day of postnatal life

(Holtzman *et al.*, 1974*a*). After this peak has been reached, there are indications that the levels fall again; i.e., the phenylalanine levels in older PKU children are lower than those of children younger than 3 years of age (Armstrong and Low, 1957). Because of this age-dependent rise in phenylalanine concentration, the earlier a PKU infant is screened, the greater the chance that the blood phenylalanine concentration will not be elevated (Holtzman *et al.*, 1974*b*) and the higher the probability that the diagnosis of PKU will be missed. Furthermore, it has been found that the time course of the increase in blood phenylalanine levels during the first week of life is different for males and females, with females showing a lag of 2–3 days before the rise (Holtzman *et al.*, 1974*b*). This sex difference, coupled with the drive for earlier and earlier screening (which, in turn, stems from the desire for the early initiation of therapy), probably accounts for the recently discovered predominance of male PKU infants over females that are detected in screening programs of newborn infants (Hsia and Dobson, 1970). It should be noted that surveys of retarded children (in contrast to surveys of all newborn infants) yield equal numbers of males and females with PKU (Jervis, 1954).

Beyond the tricky period immediately after birth, an increased phenylalanine level in the blood is a reliable diagnostic criterion for PKU or one of its variants. The fasting plasma level of phenylalanine, determined most accurately by ion-exchange column chromatography, is elevated twenty- to thirtyfold from a normal value of 0.051–0.054 m*M* (0.84 to 0.89 mg%) (Stein and Moore, 1954; Stein *et al.*, 1954; Evered, 1956; Linneweh and Ehrlich, 1962). Serum values are slightly higher (Perry *et al.*, 1967*a*). In a useful attempt to introduce a common language into this complex field, classical PKU patients have been defined as those who show persistent plasma phenylalanine levels of over 25 mg% (>1.5 m*M*) (Hsia, 1970).

As already mentioned, Fölling's original discovery of PKU stemmed not from his finding of elevated levels of phenylalanine in the blood of these patients but, rather, from his identification in the urine of an unusual product, phenylpyruvate. Since then, a long and ever-growing list of phenylalanine-derived metabolites, such as *o*-hydroxyphenylacetate (Armstrong *et al.*, 1955), phenylacetate (Woolf, 1951), phenylacetylglutamine (Woolf, 1951), and most recently, mandelate (Blau, 1970) have been found in elevated amounts in the urine and blood of PKU patients.

Subsequently, the list was shown to include metabolites of tryptophan, such as indoleacetic acid (Armstrong and Robinson, 1954) and indican (Bessman and Tada, 1960), as well as metabolites of tyrosine, such as *p*-hydroxyphenyllactic acid (Bickel *et al.*, 1955; Chalmers and Watts, 1974) and *p*-hydroxyphenylpyruvic acid (Chalmers and Watts, 1974; Hoffman and Gooding, 1969). Although it has been claimed that the *p*-hydroxy-

phenyl compounds that are excreted by phenylketonuric patients are derived in part from phenylalanine (Chalmers and Watts, 1974), a claim that would imply the presence of quite high residual phenylalanine hydroxylase activity in these patients, recent experiments with deuterated phenylalanine have shown that these parahydroxy derivatives are not derived from phenylalanine, but only from tyrosine (Curtius *et al.*, 1972*b*). As will be discussed, the evidence indicates that these disturbances in tryptophan and tyrosine metabolism are secondary to the primary disturbance in phenylalanine metabolism.

The earliest methods used to diagnose PKU followed Fölling's lead and were based on the measurement with $FeCl_3$ of phenylpyruvate in the urine. While this measurement is still useful in the diagnosis of the disease in children, it has limited value in newborns. Its limitations were pointed out as long ago as 1956, when it was shown that a PKU baby did not excrete detectable amounts of phenylpyruvate until it was 34 days old (Armstrong and Binkley, 1956). It was also shown that phenylpyruvate is no longer detectable in the urine when the serum phenylalanine levels fall below 15–20 mg/100 ml (Armstrong and Low, 1957). Both the relatively late onset of phenylpyruvate excretion and its relationship to phenylalanine blood levels have been confirmed (Rey *et al.*, 1974) and extended to the characteristics of the excretion of *o*-hydroxyphenylacetate (Rey *et al.*, 1974, Zelnicek and Slama, 1971). Despite these disadvantages and the added drawback of the nonspecificity of the $FeCl_3$ test for phenylpyruvate [*p*-hydroxyphenylpyruvate and xanthurenic acid also give a green color with $FeCl_3$ (Gibbs and Woolf, 1959)], there are still advocates of the use of measurements of urinary phenylpyruvate excretion in the diagnosis of PKU in the newborn (Allen *et al.*, 1964; Allen and Wilson, 1964).

PKU is transmitted as a recessive, autosomal trait (Jervis, 1954, 1939). The incidence of the disease is about 1 in 20,000 (Jervis, 1954; Berman *et al.*, 1969), although there is considerable geographic variation [e.g., the incidence in Ireland is only 1 in 4000 (Cahalane, 1968)]. On the basis of an incidence for the homozygous condition of 1 in 20,000, it can be calculated that the number of heterozygotes in the general population would be approximately 1 in 70.

Although heterozygotes for PKU have no signs of the disease, they can be detected by a phenylalanine tolerance test: they show a more prominent and persistent rise in plasma phenylalanine (Hsia *et al.*, 1956; Berry *et al.*, 1957) and a smaller rise in tyrosine (Jervis, 1960) than do normals. Heterozygotes for PKU can also be detected under the basal conditions; they show elevated plasma phenylalanine levels (Hsia *et al.*, 1956; Knox and Messinger, 1958) and elevated ratios of plasma phenylalanine to plasma tyrosine (Perry *et al.*, 1967b); both these deviations from the norm have

been used. These methods of detection, however, suffer from an excessive overlap of the values for the heterozygotes and the normal individuals: about 20–30% of the heterozygotes cannot be classified with certainty. The discrimination can be improved by relating the plasma phenylalanine-to-tyrosine ratio to the plasma phenylalanine concentration (Rosenblatt and Scriver, 1968). With this method, 42 of 43 presumed heterozygotes were correctly identified.

As is often the case in medicine, an effective therapy for PKU was developed long before any clear picture of the pathogenesis of the disease was available. Bickel *et al.* (1954), extrapolating from the known beneficial effects of galactose restriction in the treatment of galactosemia, treated a 2-year-old PKU child with a low phenylalanine diet for a period of 2½ years. The treatment led not only to a fall in the level of phenylalanine in the blood and urine, but also to "an appreciable improvement in the patient's mental status" (Bickel *et al.*, 1954).

This first report was quickly followed by others that described the results of treatment of an additional 11 PKU patients of varying ages with low phenylalanine diets (Armstrong and Tyler, 1955; Bickel *et al.*, 1954; Woolf *et al.*, 1955). All these early studies reported that the dietary treatment led to normalization of the clinical and biochemical symptoms. Although it was clear that the beneficial effects of the diet included relief of most of the neurological symptoms, such as seizures, the improvement in I.Q. was less dramatic. There was agreement, however, that this therapy would probably be most effective if the diet were instituted at the earliest possible age.

Subsequent experience with the diet has confirmed this early impression and has shown that there is an inverse relationship between the ultimate I.Q. of the patient and the age at which the diet had been started; early treatment leads to I.Q.s that are close to 100 (Hsia, 1970). There is evidence that beyond the age of 3 or 4 years, the diet is ineffective, which is an indication that the damage to the brain after this period of time is irreversible (Knox, 1972). Consistent with this conclusion is the evidence that the low phenylalanine diet can be discontinued after the age of about 3 years without further deterioration in mental development (Kang *et al.*, 1970*b*; Hackney *et al.*, 1968; Horner *et al.* 1962; Hudson, 1967; Vandeman, 1963). The precise date for termination of the diet is still under study.

Until 8 years ago, the effectiveness of the dietary treatment of PKU was not uniformly accepted (Birch and Tizard, 1967; Bessman, 1966). More recent results, particularly from those studies in which the I.Q. of treated and untreated (or treated late) PKU siblings were compared, show that early treatment with the diet is effective; i.e., in the vast majority of the cases, the treated sibling had a higher I.Q. (Hsia, 1970; Smith and Woolf, 1974).