Phenylketonuria Research
at the Faribault State School and Hospital
(1957-61)

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General purpose:
This research, started July 1, 1957, is now in its fourth year. It was planned in cooperation with the Pediatric Department of the University of Minnesota as a long range study, to be conducted on three different levels:

1. On the metabolic - biochemical level.
2. On the psychological level, and
3. If possible, on the genetic level.

The metabolic-biochemical and psychological studies were conducted on our P.K.U. patients in order to evaluate the effect of a phenylalanine-free or - restricted diet on older P.K.U. patients.

Four facts about P.K.U.
1. This mental condition is connected with a metabolic error. Due to the lack of a certain liver enzyme, "phenylalanine-hydroxylase", these patients are unable to oxidize phenylalanine, an amino-acid which is present to about 5% in all proteins and essential for normal growth and cell metabolism. Phenylalanine accumulates in brain and other body tissues and rises to abnormally high levels in the blood and spinal fluid of these patients. Instead of being oxidised to tyrosine, it is excreted in the urine as phenyl pyruvic acid. The phenylpyruvic acid gives a blue color when 10% Ferric Chloride is added to the urine (Pollings test 1934).

The presence of excessive phenylalanine and its abnormal metabolite: disturbs also the normal pathways of other amino-acids, notably of Tyrosine and Tryptophane. Such a blockage of tyrosine oxidation causes a deficit in melanin and the light blond hair and blue eyes in most of our patients. A blockage in the tryptophane pathway (on the step of decarboxylation of 5-Hydroxy tryptophane) results

* P.K.U. - Phenylketonuria

in low Serotonin (5-hydroxy-tryptamine) blood levels. The importance of Serotonin for normal brain metabolism and mental processes is now recognized, Many investigators feel that in this . Serotonin deficiency we might have the key for the mental retardation in P.K.U.

Genetic aspect

P.K.U. belongs to the group of so-called ''Inborn errors of Metabolism". Through pedigree studies of affected families (Foiling, Penrose) and statistical analysis of large numbers of sibships (Jervis) , it has been established that P.K.U. is inherited through a single autosomal recessive gene»

Diet studies with phenylalanine restricted diet

The diet therapy was inaugurated in December 1951 by Bickel in Burmingham, and independently in early 1952 by Armstrong in Salt Lake City. Since then, it has been given in many Medical Centers, foremost in U.S.A., England and Germany, all together to about 80 children. It is now general consensus that mental retardation can be prevented, when P.K.U. is diagnosed early in infancy and these children placed on the phenylalanine-free or restricted diet before 16 months, better still within the first 2 months of life (LaDue) . Knox has shown in a recent excellent review of all treated and published cases, that 5 point© in the final I.Q. are lost for each 10 weeks of delayed treatment.

Artificially produced P.K.U. in infant monkeys

This is probably the most dramatic recent development in P.K.U. investigation: Br. H. Haisman, pediatrician in Madison, Wisconsin, reported on the 1st International Medical Congress on Mental Retardation in Portland, Maine, July 1959, that feeding of large doses of L-phenylalanine (3.0 rag per kg per day) to infant Rhesus monkeys reduced high phenylalanine blood levels and positive urines for phenylpyruvic acid. The monkeys are tested regularly for their behavior, social interaction and intellectual accomplishments. These monkeys act definitely retarded.

III Procedures used;

In 1957 the laboratory and chemical methods were established. The diet studies were started in Jan. 1958. This is a controlled study. Patients on the diet are matched with untreated patients of the same age, sex and intelligence level as controls. Both groups are followed in regular intervals in regard to their biochemistry, clinical signs, behavior, intelligence and EEG. The ages of our diet patients range from 3 to 46 years with predominance of the younger age group. In 1958, 6 patients were placed on the diet, in 1959 we added 3, in 1960 one more patient. In total we have treated 10 patients. Two of our diet patients were taken over from Dr. Berendes' diet group at the University of Minnesota (fall 1959) .

Five of the patients received the diet for 3 years (or a little less) , two for 2 years, one for 16 months, one for ½ year and one for 3 months. Two of our patients (age 10 and 19) were taken off the diet after 6½ and 16 months respectively, when they created repeated feeding difficulties or showed severe regurgitation and apparently derived no appreciable benefit from the diet. In 1960, we terminated the diet for our 2 oldest patients (age 38 and 46 yrs.) after 2 and 2½ years respectively. One 12 year old boy was taken off the diet on his 15th birthday. Presently we still have 5 patients on the diet, i to 10 years old,

The phenylalanine-free food used in our diet studies is an artificial food product prepared from milk casein. After acid hydrolysation of the casein, the phenylalanine is eliminated by absorption on activated charcoal and replaced by other amino-acids. Three food products of this type are available on the market: One product by Allen & Hanbury, London; "Ketonil" by "Merck, Sharp and Dohme, and "Lofenalac" developed by Mead-Johnson. We have used Lofenalac in our studies.

The Lofenalac diet is easily prepared and generally well taken by the patients, either in form of a formula or as a pudding or fruit jello with different kinds of flavors. The patients show satis-

factory weight gain and look well when the "Lofenalac diet" is supplemented by vegetables, fruits and other sources of natural proteins within the permitted tolerance of phenylalanine intake.

Specific aims:

In our studies we tried to answer the following questions;

1. Is the phenylalanine-free diet effective also in older patients?
2. How long does this diet have to be given to become effective?
3. Which of the biochemical, clinical, psychological and EEG changes are reversible in older P.K.U. patients?
4. Is the mental retardation due to a permanent and irreversible brain damage sustained in the formative stages of early infancy, or is it due to a toxic and reversible action of phenylalanine and some of its metabolites which interfere with the normal metabolism and function of the brain?

results with the phenylalanine-restricted diet:

In over 3 years of diet studies we have gained the following impressions:

1. Under phenylalanine-free or - restricted diet the metabolism and biochemistry of older P.K.U. patients becomes normal. They react to the diet in a similar way as do infants and small children. The high blood-phenylalanine values fall to normal levels and the urine becomes negative for phenylpyruvic acid.
2. There appear, without doubt, favorable somatic changes. We have seen improvement in skin manifestations and neurological symptoms Better coordinated movements and motor ability in some, decrease in muscular rigidity in others. A 7 year old boy who was only able to crawl, started to stand up after 4½ months and to walk after 7 months on the diet.
3. The electroencephalograms of the 10 diet patients showing various pathological patterns, were checked in 3 months intervals: Only 2 (G. v. and M. M.) have shown changes under the diet, and these only transitory. The rent of the EEG'S regained entirely unalter
4. In 8 of our 10 patients we have also seen some personality change They improved in their disposition, became leas tense, less

irritable and hyperactive, improved in alertness and attention span. But only two school boys, ages 7? and 12, showed an increase in their M.A. by 4- and 6 months respectively while on the phenylalanine-free diet for 3 years. This gain is practically insignificant, especially when control P.K.U. patients, not on the diet, show similar gains.

An interesting observation was made in regard to the somatic and behavioral changes: In 4 of our patients (Gr. W., M. McH., J.S., D. M,) the greatest gains occurred during the first few weeks and months of the diet: Rigidity, irritability, apathy, sluggishness and listlessness gave way to relaxation, better mood, smiling expression, to alertness and cognizance of people and surroundings. After that, progress seemed to slow down or come to a standstill. If originally we had expected to arrive at a clearcut answer in regard to reversibi1ity of symptoms and mentality, we realize now that, possibly, we are confronted here , with two etiological components in the picture of P.K.U: A reversible "toxic" component associated with the faulty metabolism of phenylalanine, and an irreversible "organic component linked with the permanent brain damage and developmental arrest in early life. In other words: By giving the phenylalanine-free diet, it is possible to correct the faulty metabolism of phenylalanine and other aminoacids and to ameliorate the associated clinical signs of P.K.U. But it is impossible to undo whatever structural brain damage has occurred through the prolonged action of the same metabolic factors. Similar views have been expressed by Armstrong (1957) and by Woolf and co-workers (1957)•

results of basic research on the metabolic and biochemical level:

1. There exists a tolerance level for the daily phenylalanine intake. This varies from person to person, but apparently also with age. In infants it is reported to be between 20 to 25 mg. per kg. body-weight (Hsia and Berendes) . We confirmed that in a 3 year old boy. we found it between 12 and 20 mg. per kg. at school age and 7 to 11 mg. per kg. in adults.
   There is an interesting fact connected with this tolerance level:

Independent from age or bodyweight, the P.K.U. patient is apparently not able to tolerate more than about 400-500 mg phenylalanine per day. This appears to be the critical amount which the damaged phenylalanine hydroxylation enzyme system is able to cope with. It is about a tenth of the daily intake in normals.

2. Intermittent Excretion A most interesting phenomenon is that of the "intermittent excretion". When we surveyed our institutional population, we found a certain number of P.K.U. patients positive who had been negative in earlier years. At first, we interpreted these as "false negative" tests either due to faulty test methods or done on samples having been exposed to bacterial action at room temperature. Wie considered also the possibility that the urines had been tested at a period of low protein and phenylalanine intake. But a certain number of our P.K.U. patients show intermittent excretion quite frequently. Wright and Tarjan have described 2 such cases, Armstrong 1. Centerwall estimates that 5-10%, Lyman that 20% of all P.K.U. patients show intermittent excretion.

   In 6 of our diet patients we were able to demonstrate, that they became "intermittent excreters" when their blood-phenylalanine level stayed at the threshold level of 15-2 0 mg %.

3. Comparative determinations of phenylpyruvic acid in the urine, both with the Dinitrophenyl-hydrazine method (Penrose and Quastel) and the colorimetric ferric citrate method (Kropp and Lang) show that some of our patients at times excrete an unknown Keto-acid, other than diacetic acid and acetone. Such a Keto-acid has been observed by Woolf and Berry (1952) .

4. In early 1959 we surveyed our entire population of 3,298 patients for P.K.U. In this survey we used the new Phenistix method (Ames Co .) - and compared it with the standard ferric chloride test of Foiling. We found it entirely satisfactory and can recommend it strongly for general use in offices, hospitals and well-baby clinics. It is faster and simpler to perform, it needs only a few drops of urine on a wet diaper, it is easier to interpret and permits even a quantitative estimate. This quantitative estimate compares very well with the quantitative chemical determination, done simultaneously on 780 urine samples.

VII Pilot Studies on the genetic level:

Phenylketonuria is an inherited disease due to a single autosomal recessive gene. Two entirely normal parents may carry this abnormal genie. According to the Mendelian laws of inheritance, 25% of their children should be mentally retarded, 25% normal and free of the abnormal gene, while 50% of their children, though appearing normal, should be carrying the abnormal gene and Would be regarded as "heterozygous carriers".

It has become possible to find the carriers in the families of phenylpyruvic patients by phenylalanine-tolerance tests. (Hsia, Paine and Driscoll; Berry, Sutherland and Guest: Jervis; Haisman; J. Anderson and co-workers) .

After developing chemical and paperchromatographic micromethods for phenylalanine and tyrosine determination, we did P. A. tolerance tests on several normal individuals, on two "heterozygous carriers" and on two P.K.U. patients, We found the expected differences described in the literature,

We have not proceeded with these tests, since the dosage of the test load is still under discussion. Hsia had originally proposed 0,1 gm of L-phenylalanine per kg,, Jervis lateron 0.3 gm per kg, But Anderson found very undesirable side effects using these large quantities, and proposes now a medium dose of 0.2 gm per kg,

We might enter this field of genetic studies and of determination of the heterozygous carriers in our P.K.U. families on a later date, in cooperation with the newly established genetic unit of the Minn, State Board of Health.

VIII Conclusion:

1. 3½ years of diet studies on older P.K.U. patients have shown that they react favorably to the reduced phenylalanine intake in their biochemistry and that they show somatic and behavioral improvements, We consider this area as the reversible component of P.K.U., due to toxic effects of the disturbed aminoacid metabolism. But the organic brain damage, caused by the same

metabolic factors in the early stages of brain development are permanent and irreversible, as shown by the unaltered EEGs and objective intelligence tests.

2. These results make it imperative that P.K.U. is diagnosed in early infancy and the P.A. free diet started, if possible, within the first 2 months of life. It is general concensus no?/, that the diet has to be given for a minimum of 5 years.

3. In order to recognize the 10 or 20% of intermittent excreters, the urines of all infants should be tested repeatedly at the age of 6 weeks, 2 months, 6 months and 9 months, either with the 10% ferric chloride diaper test or with the phenistix strip method.

   Special attention should be given to the children of the following high risk groups:

   a. All children with neurological disorders, convulsions and eczema, possibly also their siblings.
   b. All mentally retarded children and their siblings.
   3. All infants of nearer and distant relationship to known P.K.U. patients.

4. All families of P.K.U. patients should be informed about this metabolic error and its relationship to mental retardation. They should be alerted to the fact that mental retardation in these cases can be prevented when the condition is diagnosed in early infancy. Mothers of P.K.U. patients in the childbearing age should be alerted to the possibility that 25% of her children born subsequently might inherit the condition. All her children should be tested for P.K.U. routinely and repeatedly.

   We have contacted several families of our P.K.U. patients, and have informed them about the conditions. We plan to expand this type of educational program for the families of all our P.K.U. patients.

Anticipated Research Program

for the budget year 1961-62

We propose to conduct the research during the coining budget year in three fields:

I. Phenylalanine free-diet studies

though we consider the goal of our diet studies accomplished in the main points, still we would wish to continue the diet on 5 of our younger patients (ranging from 3 to 10 years of age) as long as possible, since they are showing some encouraging personality changes and neurological improvement. Dr. H. Biekel, who introduced the P.A. free diet in 1951, felt that the diet should be continued for 6 to 8 years or until puberty is reached (1st Int. Congress on Mental Retardation, Portland, Main, July 1959)

II. We propose to continue the biochemical laboratory for the following purposes:

A. To maintain close biochemical control of the patients on the phenylalanine free diet by regular follow-up of their blood phenylalanine level and weekly urine chemistry.

B. To continue basic research in the area of Phenylketonuria in regard to the following important problems:

1. Variations of blood Phenylalanine levels in untreated P.K.U. patients.

2. To investigate further certain P.K.U. cases who are showing "intermittent excretion" of phenylpyruvic acid.

3. To continue the investigation of the renal threshold by studying she "renal clearance" of phenylalanine, phenylpyruvic acid and other amino acids.

III. A. We propose to extend our basic research into other areas of Inborn Errors of Metabolism and pay attention to other types of Mental Retardation present in our institution including Galactosemia, Gargoyilsm and De Toni - Fanconi syndrome.

B. We plan to do paper-chromatographic screening tests for aminoaciduria by the method of Dr. Ghadimi (Boston) on certain types in our population: patients with cataract, microcephaly, etc., as outlined in a progress survey by Richard S. Paine, Boston 1960.

C. We propose to investigate the tyrosine and tryptophane metabolism of mongolism.

Pilot studies done by Dr. John Anderson, red. Dept. of U of M, on mongolism have shown that mongoloids have apparently a lower excretion of 5-hydroxy-indole-acetic acid and of 3-hydroxy-kynurenic acid, 3-hydroxy-anthranilic acid and catechol. These chemical bodies can be determined by paper chromatography, Dr. J. Anderson has proposed that we should use our laboratory facilities to extend these investigations.