Studies on Phenylketonuria. V. Observations on a Newborn Infant with Phenylketonuria.* (22775)

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Phenylketonuria, an inherited biochemical abnormality in the oxidation of phenylalanine to tyrosine, is usually associated with mental deficiency(1). Some of the pathological symptoms may be due to the presence of some toxic substance formed as a consequence of the greatly increased amount of phenylalanine which accumulates in patients(2-5). It was important to determine whether newborn infants with phenylketonuria are biochemically normal, since the rate of development of biochemical abnormalities might relate both to the age of onset of symptoms and to the severity of the mental defect observed in later life. There have been indications(5,6) that the urine of newborn infants later found to have phenylketonuria gives a negative reaction for phenylpyruvic acid when tested with ferric chloride. This test is unreliable, however, in establishing the absence of phenylpyruvic acid from dilute samples of urine such as those excreted by small infants, and, in order to determine the degree of biochemical abnormality, it is necessary to use other tests. These are the 2,4-dinitrophenylhydrazine test for phenylpyruvic acid, paper chromatography of an extract of urine in order to detect phenylpyruvic, *o*-hydroxyphenylacetic(7), *p*-hydroxyphenyllactic and indolelactic acids, and determinations of serum levels of phenylalanine. Arrangements were made to obtain blood and urine from infants born to parents who had previously had a child with phenylketonuria. The third infant studied in this manner was phenylketonuric.

Methods. Cord blood was collected immediately after parturition; blood from the newborn infant was collected within 3 hours of birth and at intervals throughout the first month. Serum phenylalanine was determined by a modification(4) of the method of Kapeller-Adler. Urine was collected as frequently as was feasible throughout the first month; unfortunately, the infant observed was a girl, it was impractical to hospitalize her, and urine samples were small, though sufficient for these studies. For qualitative test, the dinitrophenylhydrazine reagent was added to an equal volume of urine; a positive test is shown by the appearance of a yellow turbidity at concentrations of phenylpyruvic acid as low as 2 mg %. This test has proved to be far more reliable than the ferric chloride test for phenylpyruvic acid. Urine was extracted by the procedure described earlier(8); relatively larger volumes of solvents than usual were required because of the small amount of urine available, but the final ethyl acetate solution of the organic acids was conc. in a stream of air and the final volume was adjusted by the addition of ethyl acetate. Small amounts of phenylpyruvic acid may be detected on 2-dimensional chromatograms by the development of a brown color with ammoniacal $AgNO_3$(9); larger amounts are evident as a diffuse pink area on chromatograms which have been sprayed with diazotized sulfanilic acid(12) (phenylpyruvic acid, R_F 0.58 in isopropyl alcohol-aqu. $NH_3 - H_2O = 8:1:1$ (Ipr – NH_3) and 0.66 in benzene-propionic acid-water = 2:2:1 (organic phase) (Bz-prop.). *o*-Hydroxyphenylacetic acid was estimated as described previously(8), indolelactic acid by spraying 2-dimensional chromatograms with *p*-dimethylaminobenzaldehyde (purple color, R_F 0.48, Ipr-NH_3, and 0.38, Bz-prop.) and *p*-hydroxyphenyllactic acid with diazotized sulfanilic acid.

Clinical summary. Baby M was born at

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term of an uneventful pregnancy; birth weight, 5 lb. 12½ oz. She was completely normal in all respects when followed in the hospital during the newborn period and when examined at the age of one month. She was breast fed and received adequate nourishment. An electroencephalogram, made at the age of 33 days showed evidences of abnormalities. These included isolated spikes and some spike and slow wave complexes of irregular contour, observed in both the waking and sleep records. The record was suggestive of a diffuse cortical disturbance, which appeared to be more marked over the right hemisphere. In order to avoid possible damage to the child, the studies were not continued after phenylpyruvic acid appeared in her urine. She was brought into the hospital at the age of 33 days, and at the age of 40 days she was started on a phenylalanine-deficient regimen.†

Results. The results of the serum phenylalanine determination are shown in Fig. 1; for comparison, values are included from the 2 infants studied previously who proved to be normal. The slow gradual rise of the phenylalanine level in Baby M is in contrast to the fairly rapid (1 day) rise to high levels in older patients with phenylketonuria who were maintained at normal blood phenylalanine levels by a phenylalanine-restricted regimen and then given a natural diet(4). The chromatographic study of the excretion of the minor abnormal metabolites, *o*-hydroxyphenylacetic, indolelactic, and *p*-hydroxyphenyllactic acids showed that, at 5 days of age and with a phenylalanine level of 24 mg %, the infant excreted *none* of the abnormal metabolites always observed in older phenylketonurics. The next urine sample (16 days of age, serum phenylalanine, 47 mg %), contained greatly increased amounts of the above acids, although the rate of excretion of *o*-hydroxyphenylacetic acid was only one-fifth of that observed in older patients

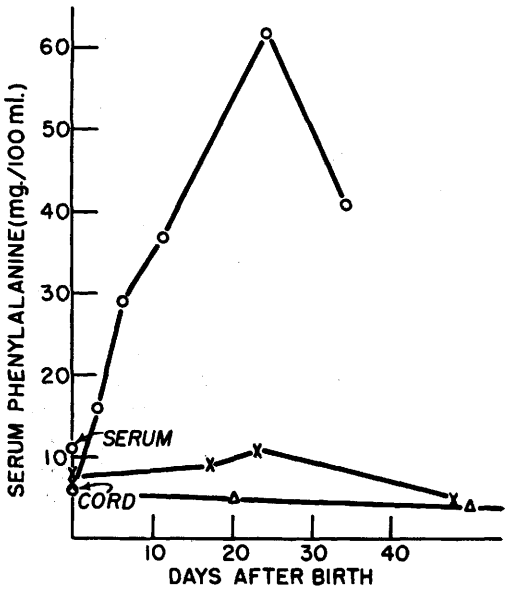

FIG. 1. Serum phenylalanine levels in newborn infant with phenylketonuria. ○, Baby M. Serum sample drawn just after birth was badly hemolyzed. A higher value was obtained than with cord blood, probably because of interference with the normal chromogen. ×, Baby U; △, Baby L.

with a comparable serum phenylalanine level. The rate of excretion of *o*-hydroxyphenylacetic acid then remained at about one-fifth that observed in older patients.

The most striking finding was that the baby did not excrete a detectable amount of phenylpyruvic acid until it was 34 days old. At this time the urine contained no more than 0.2 mg phenylpyruvic acid per mg creatinine and the daily excretion could have been no more than 7 mg. The excretion of phenylpyruvic acid by most patients is 1.5-3.0 mg/mg creatinine. Phenylpyruvic acid also was clearly detectable for the first time on a 2-dimensional chromatogram of the organic acids extracted from this sample of urine.

Discussion. The possible absence of phenylpyruvic acid from the urine of phenylketonuric infants before the age of one month calls for caution in arriving at a negative diagnosis based on testing during the newborn period the urine of siblings of older children with phenylketonuria. A determination of serum phenylalanine would provide a more reliable test. Although the present report is

† The diet was similar to that reported previously (4), with the exception that a phenylalanine-deficient casein hydrolysate, supplemented appropriately with amino acids, was used in place of the amino acid mixture.

of a single case, the delay in the development of typical biochemical abnormalities is consistent with previous observations that some phenylketonuric children may develop normally for a few months before developing pathological symptoms(4,5). It might be expected that further observation on other newborns with phenylketonuria will reveal considerable differences in the time required for phenylpyruvic acid to appear in their urine.

The delayed appearance of phenylpyruvic acid might be explained most simply by the hypothesis that phenylalanine transaminase is not present in newborn infants with phenylketonuria, and that the production of phenylpyruvic acid and the accompanying fall in serum phenylalanine level indicate the maturation of this enzyme system. A similar development of the tyrosine oxidase system has been shown to occur around the time of birth (10).

Summary. Biochemical studies have been performed on a phenylketonuric infant from birth to the age of 34 days. Cord blood and newborn infant blood contained normal levels of phenylalanine, the serum phenylalanine rose to 62 mg % at 24 days, and decreased to 41% at 34 days. Phenylpyruvic acid was not excreted in detectable amounts until 34 days. *o*-Hydroxyphenylacetic acid was not present in excess at 5 days, but from 16 to 34 days was excreted at about one-fifth the rate usually observed in phenylketonuria. The minor abnormal metabolites indolelactic and *p*-hydroxyphenyllactic acids were not present at 5 days, but were present at 16 days.

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