

Aminoacidemia of Prematurity

Its Response to Ascorbic Acid

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THE CLASSIC studies of Levine, Gordon, and Marples^{1,2} demonstrated a defect in aromatic amino-acid metabolism of premature infants receiving a high protein, low ascorbic acid diet. Application of screening studies for detection of phenylketonuria in newborn infants led to the observation of elevated levels of blood phenylalanine, particularly in premature infants. The phenylalanine elevation was associated with high blood levels of tyrosine. This metabolic peculiarity of prematures assumes practical import, for (a) the abnormal blood values may be confused with the blood findings of phenylketonuria and (b) this aberration of amino-acid metabolism may contribute directly or indirectly to the poor intellectual development of the prematurely born child. The present studies were undertaken to permit serial measurement of phenylalanine and tyrosine levels in blood from premature infants and to clarify the role of ascorbic acid in aromatic amino-acid metabolism.

Material and Methods

Subjects.—All infants born at the Cincinnati General Hospital between Feb 2 and July 20, 1965, weighing less than 2,268 gm (5 lb) were studied. The infants were admitted to the "low-birth-weight nursery" which had an average daily census of 18 infants (range 14 to 25 infants). The routine diet consisted of one third evaporated cow's milk, two thirds water, and additional carbohydrate calculated to result in a caloric content of 20 calories/oz. The estimated protein content was 2.3 gm/100 cc and the ascorbic acid content of the terminally sterilized formula was negligible.* On occasion low-protein feedings or dextrose-water were utilized. The mean protein intake was about 5 gm/kg/24 hr. Infants were discharged when they achieved a weight of 2,260 gm (5 lb).

The testing was divided into three periods. During Period 1 (Table 1) all infants received in their formula a supplemental daily dose of 100 mg of ascorbic acid. This was begun late in the first week

of life. During Period 2, large supplementary doses of ascorbic acid were no longer administered to infants newly admitted to the nursery. Occasionally infants received 10 to 15-mg daily doses of ascorbic acid. Infants remaining in the nursery from the previous high ascorbic-acid period continued to receive daily doses of 100 mg of ascorbic acid. During Period 3, additional infants with elevated serum levels of phenylalanine and tyrosine were observed. Eleven infants with elevated serum-amino-acid levels received a single intramuscular dose of 25, 50, or 100 mg of ascorbic acid.

Collection of Specimens.—Beginning Feb 2, 1965, and at weekly intervals thereafter, capillary blood specimens for serum phenylalanine and tyrosine determinations were obtained from all infants. During Period 1 (the 14-week period from Feb 2 to May 4) weekly capillary blood specimens were obtained from 70 low-birth-weight infants receiving 100-mg doses of ascorbic acid daily. An additional 61 weekly blood specimens were obtained from 18 of these infants who were continued on large doses of ascorbic acid beyond this date. During Period 2 (the 11-week period following May 4) 150 weekly blood specimens were obtained from 59 low-birth-weight infants receiving daily doses of 0 to 15 mg of ascorbic acid.

During Period 3 (from July 21 to Dec 7) 21 infants who had not received supplemental ascorbic acid had serum-tyrosine levels above 10.0 mg/100 cc. Eleven of these infants received a single intramuscular dose of ascorbic acid. Seven patients received 50 or 100 mg of ascorbic acid, and four patients received 25 mg of ascorbic acid. Blood was obtained from these infants by capillary heel-stick immediately prior to the administration of ascorbic acid, and 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 18 to 20 hours, and 24 hours after the administered dose. Random urine specimens collected from these infants before and after the administration of ascorbic acid were examined by routine chromatographic methods for the tyrosine metabolites *p*-hydroxyphenylacetic acid, *p*-hydroxyphenyllactic acid, and *p*-hydroxyphenylpyruvic acid. Additional random urine specimens obtained from infants before and after the administration of ascorbic acid, and from infants receiving daily doses of 100 mg of ascorbic acid, were assayed for 5-hydroxyindoleacetic acid.*

Methods.—Blood was collected by capillary heel-stick in two microhematocrit tubes (50 μ l capacity). Serum was separated in a high-speed microcentrifuge and drawn into a graduated micro-

* The average vitamin C content of reconstituted evaporated milk is approximately 6 mg/liter.

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TABLE 1.—Ascorbic Acid Intake

	Period 1 (Feb 2— May 4)	Period 2 (May 5— July 20)	Period 3 (July 21— Dec 7)
No. infants	70	59	11
No. specimens	225+61*	150	88
Ascorbic acid supplement	100 mg/day	0-15 mg/day	25, 50, or 100 mg single I.M. dose
Protein in formula	2.3 gm/100 cc	2.3 gm/100 cc	2.3 gm/100 cc
Protein intake/24 hrs	5 gm/kg	5 gm/kg	5 gm/kg

* Specimens obtained from 18 infants who continued to receive 100 mg/day of ascorbic acid during Period 2.

During Period 1, newly admitted infants received 100 mg of ascorbic acid daily. During Period 2, ascorbic intake was limited to 0 to 15 mg daily. During Period 3, some infants with elevated blood tyrosine and phenylalanine levels were studied following administration of single doses of ascorbic acid.

period of high ascorbic acid intake the weekly incidence of infants with serum tyrosine levels greater than 10.0 mg/100 cc was usually zero and never more than 7% (Fig 1).

Of 150 serum tyrosine levels obtained at weekly intervals from 59 low-birth-weight infants receiving 0 to 15 mg of ascorbic acid daily, 20% of the specimens (29 specimens) had levels higher than 10.0 mg/100 cc, with 10% of the levels above 20.0 mg/100 cc. Serum tyrosine levels above 10.0 mg/100 cc on one or more determinations were found in 32% of the infants (19). The increased frequency of infants with elevated serum tyrosine levels in the low ascorbic acid intake group is highly significant (χ^2 with Yates correction = 10.0 P is less than 0.01). The frequency in a given week of serum tyrosine levels greater than 10.0 mg/100 cc increased from 0%-7% to 20%-25% during the period of low ascorbic acid intake (Fig 1).

Serum Phenylalanine (Periods one and two).—Of 286 weekly serum phenylalanine levels obtained from 70 low-birth-weight infants receiving 100 mg of ascorbic acid daily, less than 1% of the specimens (two specimens) had levels above 5.0 mg/100 cc. Of the infants, 3% (two) had one serum phenylalanine level above 5.0 mg/100 cc.

Of 150 weekly serum phenylalanine levels obtained from 59 low-birth-weight infants receiving 0 to 15 mg of ascorbic acid daily, 10% of the specimens (15 specimens) had levels higher than 5.0 mg/100 cc, with a single level of 10.5 mg/100 cc being the only value higher than 10.0 mg/100 cc. Of the infants, 20% (12) had serum phenylalanine levels higher than 5.0 mg/100 cc on one or more determinations. The in-

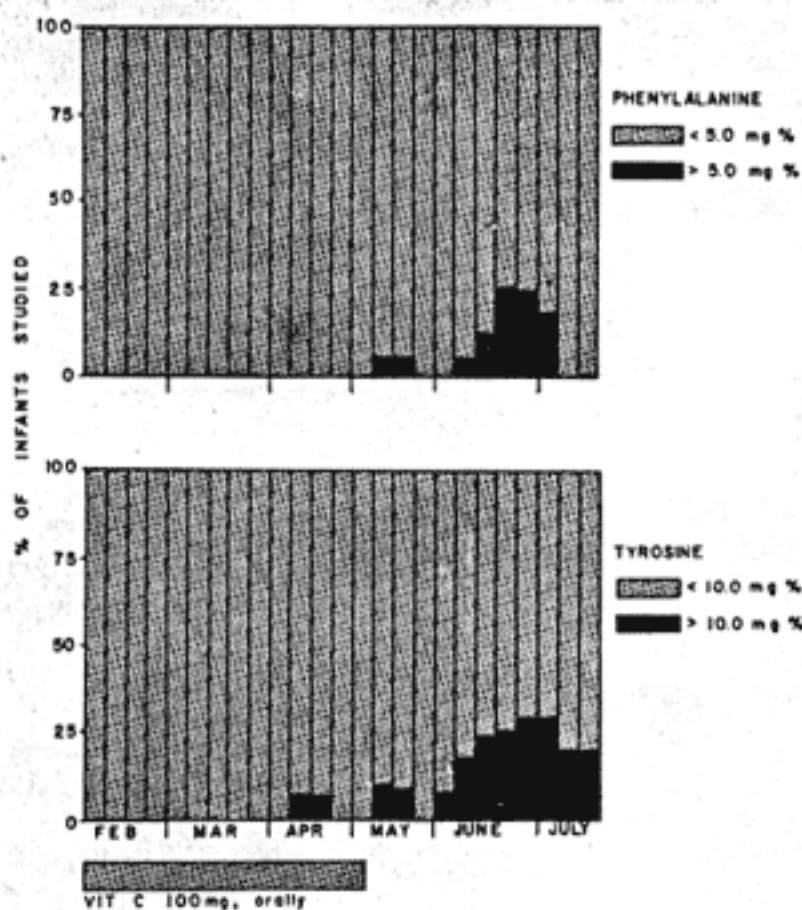


Fig 1.—All infants in the low-birth-weight nursery (less than 2,270 gm [5 lb]) were studied each week. The percentage of infants with abnormal serum-tyrosine or phenylalanine levels is indicated.

pipette of 0.025-ml capacity. Serum (20 μ l) was discharged into a clean microhematocrit tube (80 μ l capacity). Two volumes of acetone-ethanol mixture (1:1 volume/volume) were added to the serum in the microhematocrit tube. The tube was sealed at both ends and the serum was centrifuged through the acetone-ethanol precipitant. The precipitated protein was removed by snapping the tube at the scored solvent-precipitate interface. The entire supernatant was applied directly from the microcentrifuge tube to an 11-inch (Whatman No. 1) filter paper. The filter paper was placed upright in a chromatography jar containing a solvent mixture of 120 ml of butyl alcohol, 30 ml of glacial acetic acid, and 30 ml distilled water. The chromatogram was allowed to remain in the solvent overnight. The dried chromatogram was sprayed with ninhydrin reagent (0.2% in water-saturated butanol containing 5% ethanol) and heated to 90 to 100 F for ten minutes. The density of the phenylalanine and tyrosine spots was read using a Photovolt Densitometer fitted with a 545 microfilter, and were compared to chromatograms of standard solutions containing amino acids representative of plasma.

Results

Serum Tyrosine Levels (Periods one and two).—Of 286 serum tyrosine levels obtained at weekly intervals from 70 low-birth-weight infants receiving 100 mg of ascorbic acid daily, 2.5% of the specimens (seven specimens) had levels higher than 10.0 mg/100 cc. Serum tyrosine levels above 10.0 mg/100 cc were found in 9% of the infants (six). Five infants each had single determinations above 10.0 mg/100 cc and one infant had two determinations above this level. During the 14-week

TABLE 2.—Occurrence of 5-hydroxyindoleacetic Acid (5-HIAA) in Random Urine Specimens

	Premature Infants With Tyro- sinemia	Premature Infants With Treated Tyro- sinemia	Premature Infants Receiving Ascorbic Acid
No. of patients	4	4	10
No. of specimens	13	13	10
Specimens with detectable 5-HIAA levels	1	10	7
Range of detectable 5-HIAA levels ug/ml	1.04	.34-4.6	.34-2

The difference between the occurrence of detectable levels in specimens of the first group and specimens of the second and third group is significant (χ^2 with Yates correction is 12.1, P is less than 0.01).

creased frequency of infants with elevated serum phenylalanine levels during the period of low ascorbic acid intake is highly significant (χ^2 with Yates correction = 8.4, P is less than 0.01). During the 11-week period of low ascorbic acid intake the weekly incidence of serum phenylalanine levels greater than 5.0 mg/100 cc increased from 0% to 20%-25% (Fig 1).

Response to Single Dose of Ascorbic Acid (Period 3).—Seven infants with elevated serum tyrosine levels of 17 to 37 mg/100 cc and serum phenylalanine levels of 2 to 12 mg/100 cc received a single intramuscular dose of 50 or 100 mg of ascorbic acid (Fig 2). A decrease in the serum tyrosine level was noted within two to four hours after the administered dose. A maximal effect, with a decrease in the serum tyrosine levels to less than 10 mg/100 cc, was noted within 12 to 20 hours. Serum phenylalanine levels showed a similar response with an initial decrease in serum levels noted two to four hours after the administered dose. A maximal effect with decrease in the serum phenylalanine levels to less than 5.0 mg/100 cc was noted within four to eight hours.

Examination of the urine of these infants prior to the administration of ascorbic acid revealed large amounts of the tyrosine metabolites *p*-hydroxyphenylacetic acid, *p*-hydroxyphenyllactic acid, and *p*-hydroxyphenylpyruvic acid. Following administration of a single dose of 50 or 100 mg of ascorbic acid a marked decrease in the urinary excretion of these compounds occurred.

Four infants with mild to marked elevation of serum tyrosine received a single intramuscular dose of 25 mg of ascorbic acid (Fig 3). A prompt fall in both serum tyrosine and serum phenylalanine was noted within two to four hours of the administered dose. In one infant with mild serum

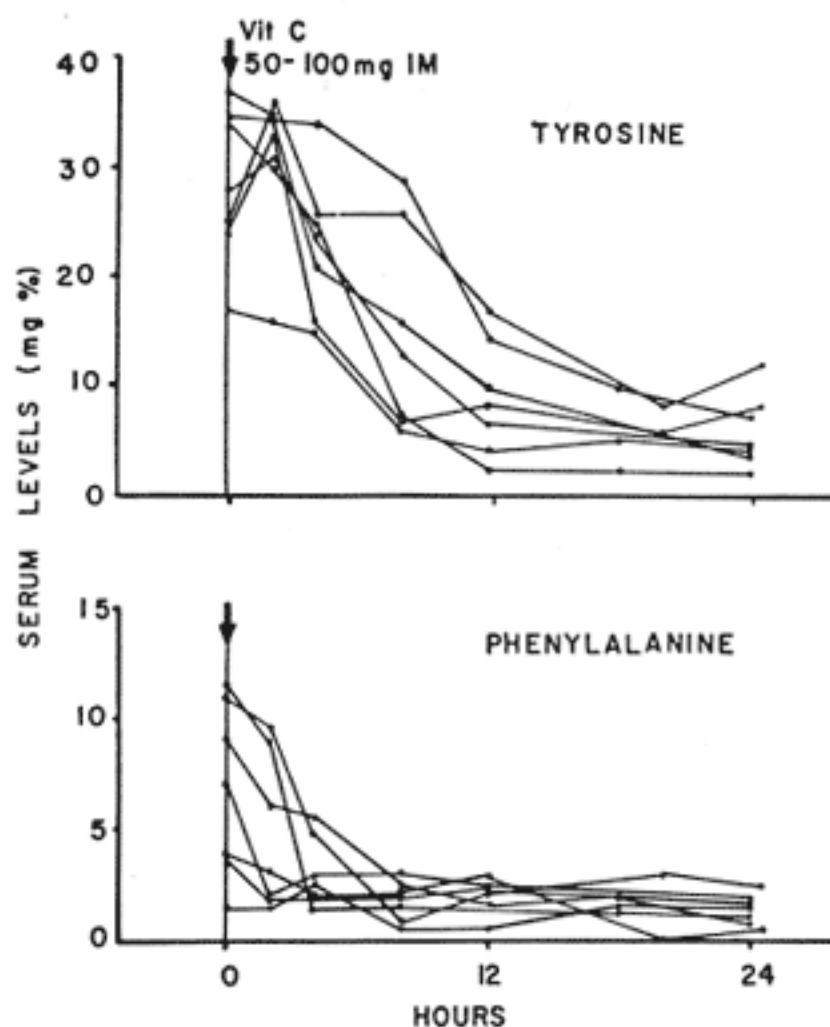


Fig 2.—Response of low-birth-weight infants with abnormal blood-amino acids to a single intramuscular dose of 50 to 100 mg of ascorbic acid.

tyrosine elevation (8.0 mg/100 cc) and in one infant with marked elevation (50 mg/100 cc) serum levels returned to normal. In the latter the response was delayed, with a decrease in the serum tyrosine level below 10.0 mg/100 cc noted 24 hours after the administered dose. In two infants with serum tyrosine levels greater than 50 mg/100 cc the slight initial decrease at two to four hours was followed by leveling off of the serum tyrosine concentration with levels of 40 to 45 mg/100 cc at 24 hours after the administered dose. Changes in serum phenylalanine levels paralleled those noted for serum tyrosine.

5-Hydroxyindoleacetic Acid.—In a few infants, 5-hydroxyindoleacetic acid was estimated in random urine specimens. Urines from the following groups of premature infants were studied: (1) premature infants with tyrosinemia who were receiving low doses of ascorbic acid or no ascorbic acid, (2) infants who had had tyrosinemia but had been treated with ascorbic acid, and (3) premature infants who had received 100 mg of ascorbic acid from late in the first week of life. The data are summarized in Table 2. The 5-hydroxyindoleacetic acid could be detected in only 1 of the 13 urine specimens from four infants with untreated tyrosinemia of prematurity. Five-hydroxyindoleacetic acid was measurable in 10 of 13 specimens from four premature infants who received ascorbic acid after tyrosinemia and in seven of ten specimens from ten prema-

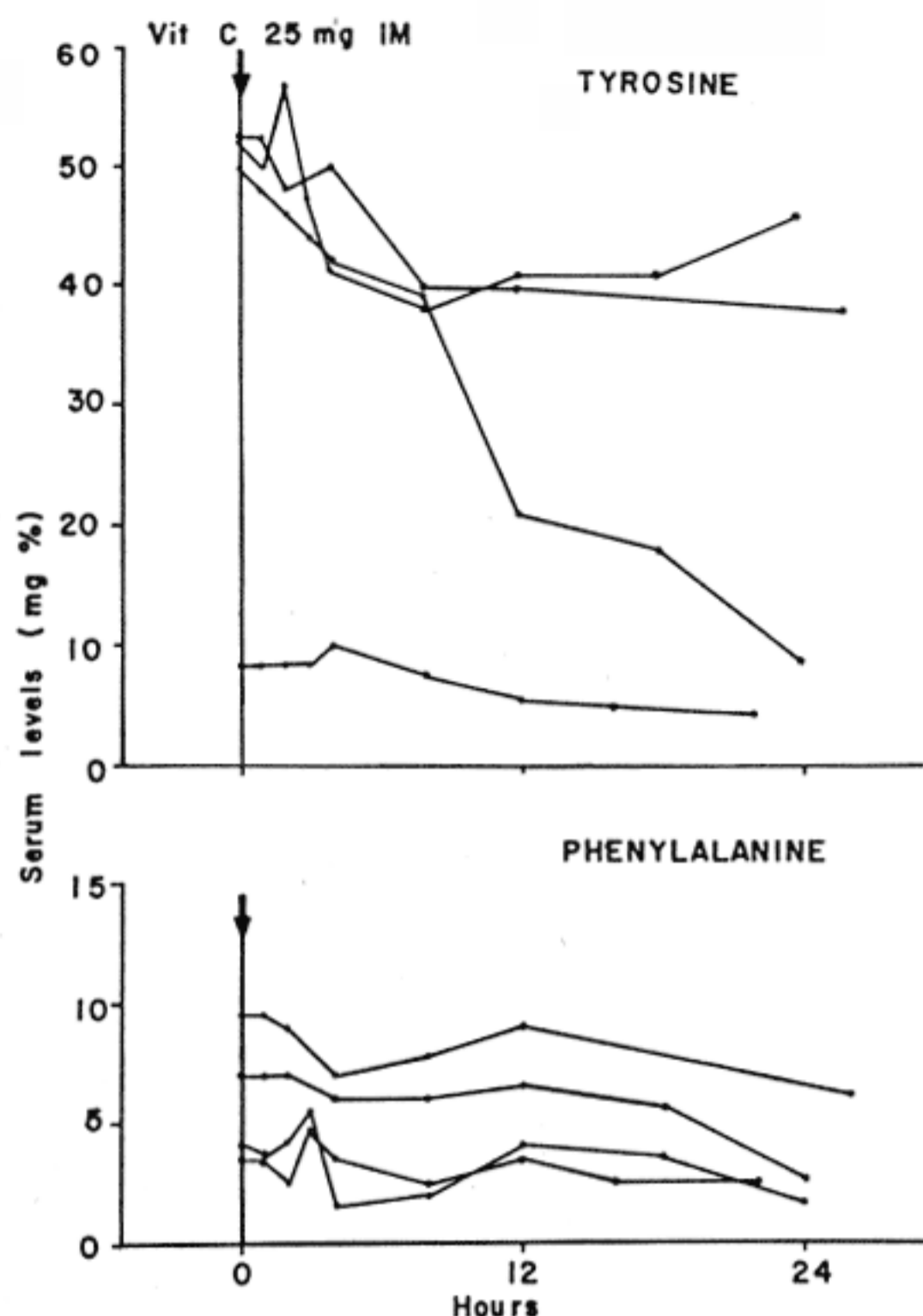


Fig 3.—Response of low-birth-weight infants with abnormal blood-amino acids to a single intramuscular dose of 25 mg of ascorbic acid.

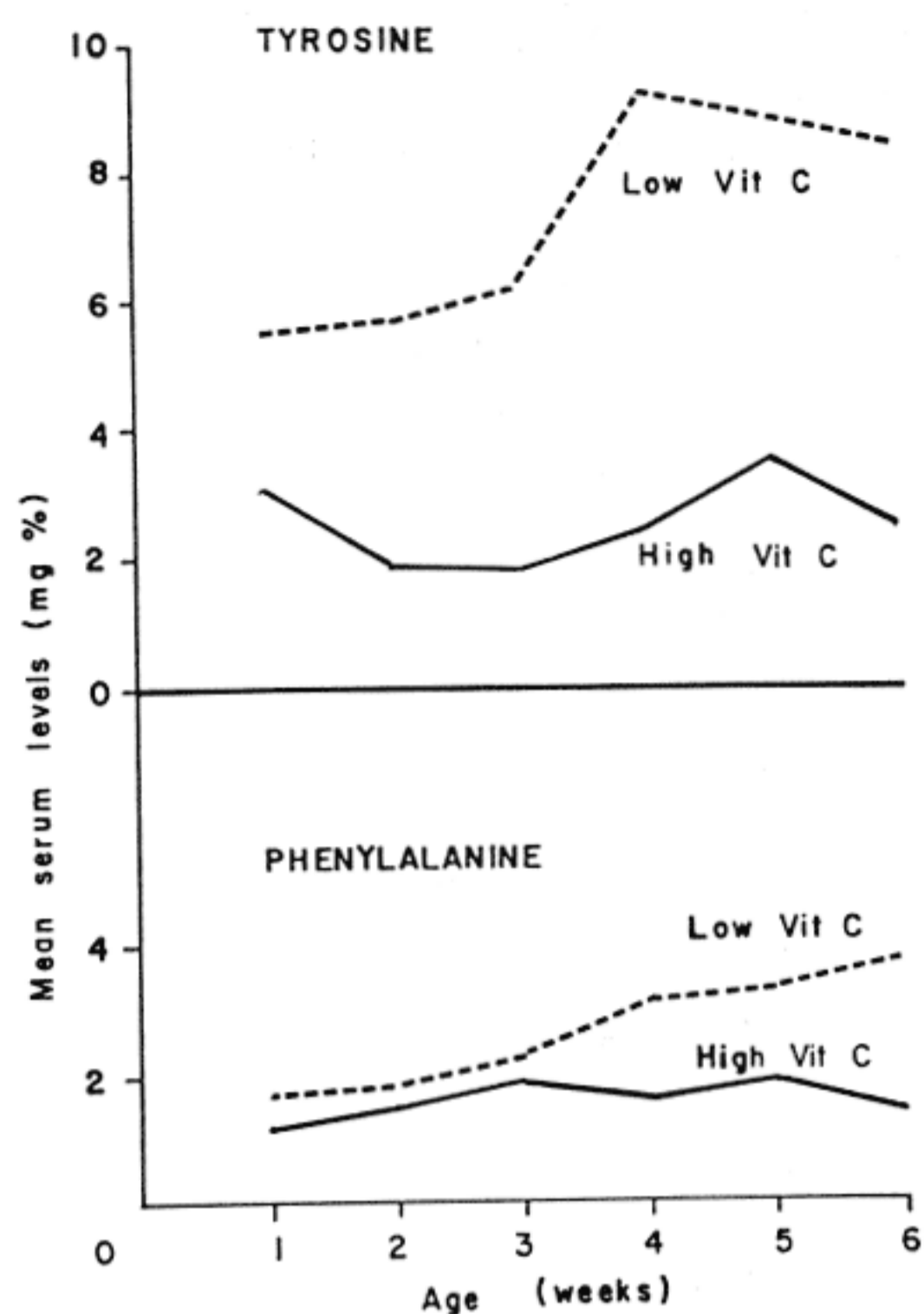


Fig 4.—Mean serum tyrosine and phenylalanine levels in low-birth-weight infants receiving the high dose of ascorbic acid (100 mg daily in formula), and the low dose of ascorbic acid (0 to 15 mg daily in the formula).

TABLE 3.—Serum-Phenylalanine and Tyrosine Levels in Infants Receiving High Ascorbic Acid Doses (100 mg Daily) and Low Ascorbic Acid Doses (0-15 mg Daily)

Age (Wk)	Serum Phenylalanine (mg/100 cc)			Serum Tyrosine (mg/100 cc)		
	Median	Mean	SE Mean	Median	Mean	SE Mean
High-Dose Ascorbic Acid						
1	1.0	1.19 (n=52)	0.13	1.7	3.05 (n=49)	0.45
2	1.5	1.47 (n=54)	0.14	1.2	1.94 (n=53)	0.36
3	1.5	1.82 (n=44)	0.19	1.5	1.83 (n=42)	0.28
4	1.5	1.57 (n=41)	0.17	1.7	2.45 (n=40)	0.43
5	1.5	1.81 (n=27)	0.28	1.6	3.54 (n=25)	1.14
6	1.5	1.36 (n=21)	0.15	1.5	2.47 (n=19)	0.68
Low-Dose Ascorbic Acid						
1	1.0	1.63 (n=48)	0.24	3.8	5.52 (n=47)	0.80
2	1.5	1.81 (n=52)	0.22	3.5	5.70 (n=50)	0.87
3	1.5	2.27 (n=43)	0.31	3.5	6.27 (n=42)	1.05
4	2.5	3.18 (n=30)	0.42	3.8	9.30 (n=30)	1.87
5	2.0	3.32 (n=14)	0.90	2.5	8.83 (n=14)	2.95
6	3.5	3.76 (n=8)	0.60	4.0	8.26 (n=8)	3.20

ture infants who received ascorbic acid from the first week of life. The difference between the occurrence of detectable urinary 5-hydroxyindoleacetic acid urine levels in the infants with tyrosinemia of prematurity and the infants in whom the condition had been treated or prevented is significant (χ^2 with Yates correction is 12.1, P is less than 0.01). The small number of patients and the preliminary nature of these data dictate caution in interpretations.

Age.—Mean weekly serum tyrosine values were calculated for each week from 1 to 6 weeks of age (Table 3). A progressive and significant difference (P is less than 0.01) was noted between the high and low ascorbic acid groups from the 1st through the 6th week of age (Fig 4). Mean weekly serum phenylalanine levels showed a similar progressive and consistent increase from the 1st through the 6th week of age, though these differences were not statistically significant.

Birth Weight.—The influence of birth weight on the occurrence of elevated serum tyrosine levels was assessed. Large infants, because they more rapidly achieved the discharge weight of 2,260 gm (5 lb), were studied for shorter periods of time. Only the first two determinations of all low-birth-weight infants (Periods 2 and 3) who had not received large doses of ascorbic acid by this age were scrutinized (Table 4). Nine of the 41 infants (22%) with birth weights of 2,000 to 2,268 gm (4 lb 6 oz to 5 lb), had serum tyrosine levels above 10 mg/100 cc during the first two weeks of life. In contrast 41% of infants (32 of 76 infants) with birth weights below 2,000 gm (4 lb 6 oz) had serum tyrosine levels above 10 mg/100 cc in the first two weeks of life (χ^2 is 4.2, P is less than 0.05).

Comment

It is 25 years since Levine, Gordon, and Marples¹ demonstrated a peculiarity in the metabolism of aromatic amino acids in premature infants receiving a relatively high protein diet (5 gm or more of protein/kg/day) without supplements of ascorbic acid. They characterized the defect by the urinary excretion of tyrosine and its metabolites *p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids. The urinary abnormalities were corrected by the administration of ascorbic acid.² The authors speculated that the etiology of the defect was related to in-

TABLE 4.—Serum Tyrosine Levels During First Two Weeks of Life

Birth Weight	Infants Studied	Tyrosinemia (>10 mg/100 cc)
More than 2,000 gm (4 lb 6 oz)	41	9 (22%)
Less than 2,000 gm	76	31 (41%)

χ^2 is 4.2; P is less than .05.

Inasmuch as the larger infants were observed for shorter periods of time, only levels determined in the first two weeks of life are included in this tabulation.

complete development of the enzyme systems involved in the breakdown of the aromatic amino acids, but favored the explanation that it occurred as a result of decreased stores of ascorbic acid in the premature infant.

In 1956, Kretchmer et al⁵ measured in vitro the tyrosine oxidation activity in fetal, newborn, and adult human liver and found the enzymatic activity to be low in the liver of prematures. More recent studies have demonstrated a decrease in the activity of tyrosine transaminase⁶ and *p*-hydroxyphenylpyruvic acid oxidase⁷ in fetal rat liver. The latter enzyme is inactivated by its substrate *p*-hydroxyphenylpyruvic acid, and the inactivated enzyme may be restored by the addition of ascorbic acid.⁸

Several investigators⁹⁻¹² have reported values for serum phenylalanine and tyrosine levels in premature infants. A moderate elevation of serum phenylalanine with levels above 5.0 mg/100 cc was described in about 25% of premature infants. A marked elevation of serum tyrosine was reported, with levels greater than 10.0 mg/100 cc noted in 25%-50% of premature infants. Serum tyrosine levels in premature infants fell within a broad range. Mathews and Partington¹² noted that the distribution of serum tyrosine levels in premature infants appeared to be bimodal, the plasma tyrosine level being less than 10 mg/100 cc or greater than 20 mg/100 cc with few values in between.

In the present study, premature infants receiving 100 mg of ascorbic acid daily did not have elevated serum levels of either phenylalanine or tyrosine. In premature infants receiving less than 15 mg of ascorbic acid daily, a moderate elevation of serum phenylalanine and a marked elevation of serum tyrosine occurred. The serum tyrosine levels were distributed over a broad range, but the bimodal distribution noted by Mathews was not confirmed. More than 25%

TABLE 5.—*Diagnosis of Tyrosinemia of Prematurity*

	Tyrosinemia of Prematurity	Phenyl- keto- nuria	Tyro- slnemia
Blood			
Phenyl- alanine	2+	4+	±
Tyrosine "Inhibition assay"	2-4+ Positive	N or ↓ Positive	4+ ±
Urine			
Phenyl- pyruvic acid	0	4+	0
O-hydroxy- phenylacetic acid	0	4+	0
P-hydroxy- phenylacetic acid	4+	±	2+
P-hydroxy- phenyllactic acid	4+	0	4+
P-hydroxy- phenyl- pyruvic acid	4+	0	4+
Ferric chloride	Positive	Positive	Positive
Response to ascorbic acid	4+	0	0
Dietary man- agement	Phenylala- nine re- striction not indi- cated	Low phenyl- alanine diet	Low phenyl- alanine, low tyro- sine diet may be of value

of low-birth-weight infants receiving low doses of ascorbic acid had serum-phenylalanine levels within the range of phenylketonuria (over 4 to 5 mg/100 cc) as measured by the widely employed "inhibition assay" screening technique.¹³ Under such conditions phenylketonuria may erroneously be diagnosed and potentially hazardous dietary control instituted.

The abnormality of prematurity may be distinguished from phenylketonuria by comparing the markedly elevated serum tyrosine levels encountered in premature infants with the normal or low serum tyrosine levels in patients with phenylketonuria (Table 5). Premature infants with elevated serum levels of phenylalanine can also be distinguished from those with phenylketonuria by observing the response to an administered dose of ascorbic acid. In premature infants a predictable rapid fall in both serum-phenylalanine and tyrosine occurs following administration of an adequate dose of ascorbic acid. Complete correction is dependent on the initial amino acid serum level and the dose of ascorbic acid administered. In the present study complete correction of the

elevated serum levels occurred following a single intramuscular dose of 50 or 100 mg of ascorbic acid. In addition, a marked decrease in the urinary excretion of tyrosine metabolites, as reported by Levine et al,² was observed in infants receiving large doses of ascorbic acid. The response to a single intramuscular dose of 25 mg of ascorbic acid varied depending on the initial serum phenylalanine and tyrosine levels.

Gentz et al¹⁴ have recently described an inborn error of tyrosine metabolism with cirrhosis of the liver and multiple renal tubular defects as in the typical de Toni-Debre-Fanconi syndrome. The disorder, designated tyrosinemia, is characterized by a lack of *p*-hydroxyphenylpyruvate oxidase. This disease can be distinguished from both tyrosinemia of prematurity and phenylketonuria by elevation of blood-phenylalanine and tyrosine and their urinary metabolites (Table 5). In tyrosinemia the biochemical abnormalities persist after administration of large doses of ascorbic acid.

Though the response to ascorbic acid in tyrosinemia of prematurity is dose-dependent, the optimal dose required to prevent this defect in aromatic amino acid metabolism remains ill defined. Mathews and Partington¹² demonstrated that the administration of 30 to 60 mg of ascorbic acid daily did not significantly influence the plasma tyrosine level in low-birth-weight infants ingesting more than 5.0 gm of protein/kg/day. However, 36% of specimens obtained from all low-birth weight infants not receiving supplemental ascorbic acid had serum-tyrosine levels higher than 10.0 mg/100 cc, as compared to 18% of specimens from infants receiving 30 to 60 mg of ascorbic acid daily. In contrast, in the present study 20% of specimens obtained from all low-birth-weight infants receiving 0 to 15 mg of ascorbic acid daily had serum tyrosine levels higher than 10.0 mg/100 cc as compared to 2.5% of specimens from infants receiving 100 mg of ascorbic acid daily. These differences appear, in part, to be dose-related.

The importance of birth weight on the occurrence of this metabolic defect has been examined. Levine et al² described increased urinary excretion of tyrosine metabolites in premature infants (birth weight less than 2,200 gm [4 lb 7 oz]) fed a cow's-milk diet. Full-term infants fed a similar diet failed to demonstrate the defect, but it could be

artificially induced by the administration of large doses of tyrosine or phenylalanine. More recent investigators¹⁵ have shown that the defect is not limited to prematures, but may infrequently be encountered in full-term infants (gestation 40 weeks or longer and birth weight greater than 2,500 gm [6 lb]) receiving a normal diet. Other investigators^{9,11,12} have suggested that infants of lower birth weights and shorter gestations tend to have higher serum-tyrosine levels. The present study confirms this impression. A significant decrease in the occurrence of elevated serum-tyrosine levels was noted in infants with birth weights of 2,000 to 2,260 gm (4 lb 6 oz to 5 lb) in contrast to infants with birth weights below 2,000 gm (4 lb 6 oz).

Several investigators have attempted to relate blood tyrosine levels to postnatal age. Menkes and Avery¹¹ noted a rise in blood tyrosine levels to a maximum of 7 to 8 days of age, followed by a gradual return to normal. La Du¹⁰ reported high blood-tyrosine levels between 1 and 2 weeks of age, values then returning to normal. In both studies the fall in blood tyrosine levels occurred coincident with the onset of administration of ascorbic acid, at 1 week of age in the former study, and at 14 days or earlier in the latter. In the present study a significant and progressive elevation of the mean serum-tyrosine levels was noted from the first through the sixth week of life. This finding is consistent with the original observations of Levine et al² who noted a defect in the metabolism of the aromatic amino acids in premature infants for as long as ascorbic acid was withheld, persisting in one infant as long as 78 days. These results suggest that this metabolic peculiarity of premature infants may persist longer than has been implied by earlier investigators.

The effect of this transient metabolic abnormality on subsequent development of the nervous system is at present unknown. Suggesting that this may be a benign metabolic curiosity are reports of patients in whom the defect was present at 3 months¹⁶ and at 49 years¹⁷ of age with no apparent ill effect on the central nervous system. In contrast to this is the report¹⁸ of a full-term infant with convulsions, spasticity, and failure to thrive, with a defect in the oxidation of *p*-hydroxyphenylpyruvic acid corrected by the administration of ascorbic acid at 3 months of age. Menkes et al¹⁹

reported 23 premature infants with transient elevation of blood tyrosine levels and 37 premature infants with normal tyrosine levels in the neonatal period. At 15 months of age no significant increase in the frequency of neurologic abnormalities was noted in the high tyrosine group, but a significant number of the larger infants had low scores as measured by the developmental intelligence quotient (IQ). The difference was not noted on follow-up examination at 18 months of age.²⁰

In humans and animals with phenylketonuria the synthesis of serotonin from its precursors is inhibited by the elevated levels of phenylpyruvic acid and phenyllactic acid. Evidence suggests that the mental defect of phenylketonuria might be the result of this associated serotonin deficiency.²¹ In the present study decreased urinary excretion of 5-hydroxyindoleacetic acid in premature infants not receiving large doses of ascorbic acid suggests a defect in serotonin metabolism in these infants. There was no demonstrable relationship between the urinary excretion of 5-hydroxyindoleacetic acid and serum levels of either tyrosine or phenylalanine. Despite the small number of patients and the preliminary nature of these data, the similarity between the damaging serotonin disturbance of phenylketonuria and the similar disturbance of premature infants not receiving supplemental ascorbic acid suggests caution in dismissing the disturbance as a harmless biochemical anomaly of prematurity.

The importance of ascorbic acid in the metabolism of the aromatic amino acids is confirmed by the findings of a moderate elevation of serum phenylalanine and a marked elevation of serum tyrosine in 25% of premature infants receiving low doses of ascorbic acid. These abnormal serum levels can be prevented and when present can be promptly corrected by the administration of large doses of ascorbic acid. In this era of routine screening for genetically determined metabolic disorders, this transient defect in the metabolism of aromatic amino acids must be distinguished from phenylketonuria with its associated inconvenient and potentially hazardous dietary management. Though this metabolic peculiarity of premature infants may persist longer than has previously been described, its effect on subsequent neurologic development remains to be evaluated.

Summary

The importance of ascorbic acid in tyrosine metabolism in premature infants has recently been questioned. The issue assumes practical import, for recognition of the deranged amino acid metabolism enters into the interpretation of screening tests for phenylketonuria. The present studies were undertaken to reexamine the findings of Levine, Marples, and Gordon which related inadequate ascorbic acid intake in premature infants to the urinary excretion of tyrosine metabolites. In addition, the present studies included blood levels of tyrosine and phenylalanine. The interrelated serotonin pathway has also been studied by measuring the urinary output of 5-hydroxyindoleacetic acid.

Marked elevation of serum tyrosine and modest elevation of serum phenylalanine occurred in 25% of low-birth-weight infants on a cow's-milk diet (2.3 gm/100 cc of protein). These abnormal blood-amino-acid levels were observed more frequently in small infants (below 2,000 gm [4 lb 6 oz]) than in large infants (2,000 to 2,270 gm [4 lb 6 oz to 5 lb]). The abnormality, if untreated, persisted as long as six weeks. It could be prevented by daily administration of 100 mg of ascorbic acid but not by smaller doses (up to 15 mg daily). It could be treated by administration of a single 100-mg dose of ascorbic acid. Distinction from phenylketonuria was possible by recognition of the elevated tyrosine levels in the premature infant and by the response of the premature to ascorbic acid. The effect of this aberration of amino-acid metabolism of premature infants on subsequent neurologic development is at present unknown.

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REFERENCES

1. Levine, S.Z.; Marples, E.; and Gordon, H.H.: A Defect in the Metabolism of Tyrosine and Phenylalanine in Premature Infants: I. Identification and Assay of Intermediary Products, *J Clin Invest* 20:199, 1941.
2. Levine, S.Z.; Gordon, H.H.; and Marples, E.: A Defect in the Metabolism of Tyrosine and Phenylalanine in Premature Infants: II. Spontaneous Occurrence and Eradication by Vitamin C, *J Clin Invest* 20:209, 1941.
3. Jeans, P.C., and Marriott, W.M.: *Infant Nutrition*, St. Louis: C. V. Mosby Co., 1947, p 123.
4. Berry, H.K., and Krupanidhi, I.: Estimation of Urinary 5-Hydroxyindoleacetic Acid by Paper Chromatography—A Sensitive Method, *Clin Chem* 11:465, 1965.
5. Kretchmer, N., et al: Certain Aspects of Tyrosine Metabolism in the Young: I. The Development of the Tyrosine Oxidizing System in Human Liver, *J Clin Invest* 35:236, 1956.
6. Kretchmer, N., and McNamara, H.: Certain Aspects of Tyrosine Metabolism in the Young: II. The Tyrosine Oxidizing System of Fetal Rat Liver, *J Clin Invest* 35:1089, 1956.
7. Goswami, M.N.D., and Knox, W.E.: Developmental Changes of *P*-Hydroxyphenylpyruvate-Oxidase Activity in Mammalian Liver, *Biochem Biophys Acta* 50:35, 1961.
8. Zannoni, V.G.: The Tyrosine Oxidation System of Liver: V. The Ability of Various Quinones to Reactivate Inhibited *P*-Hydroxyphenylpyruvic Acid Oxidase, *J Biol Chem* 237:1172, 1962.
9. Hsia, D.Y., et al: Serum Phenylalanine and Tyrosine Levels in the Newborn Infant, *New Eng J Med* 267:1067, 1962.
10. La Du, B.N., et al: A Quantitative Micro-method for the Determination of Phenylalanine and Tyrosine in Blood and Its Application in the Diagnosis of Phenylketonuria in Infants, *Pediatrics* 31:39, 1963.
11. Menkes, J.H., and Avery, M.E.: The Metabolism of Phenylalanine and Tyrosine in the Premature Infant, *Bull Hopkins Hosp* 113:301, 1963.
12. Mathews, J., and Partington, M.W.: The Plasma Tyrosine Levels of Premature Babies, *Arch Dis Child* 39:371, 1964.
13. Guthrie, R., and Susi, A.: A Simple Phenylalanine Method for Detecting Phenylketonuria in Large Populations of Newborn Infants, *Pediatrics* 32:338, 1963.
14. Gentz, J.; Jagenburg, R.; and Zetterström, R.: Tyrosinemia, *J Pediat* 66:670, 1965.
15. Bloxam, H.R., et al: An Inborn Defect in the Metabolism of Tyrosine in Infants on a Normal Diet, *Biochem J* 77:320, 1960.
16. Berry, H.K., and Sutherland, B.S.: "Tyrosinosis" in an Infant, read by Title at the Society for Pediatric Research, Swampscott, Mass, May 3-4, 1960, abstracted, *Amer J Dis Child* 100:571, 1960.
17. Medes, G.: A New Error of Tyrosine Metabolism: Tyrosinosis. The Intermediary Metabolism of Tyrosine and Phenylalanine, *Biochem J* 26:917, 1932.
18. Menkes, J.H., and Jervis, G.A.: Developmental Retardation Associated With an Abnormality in Tyrosine Metabolism, *Pediatrics* 28:399, 1961.
19. Menkes, J.H.; Chernick, V.; and Ringel, B.: Effect of Elevated Blood Tyrosine on Subsequent Intellectual Development of Premature Infants, read before the Society for Pediatric Research, Philadelphia, May 4-6, 1965, abstracted, *J Pediat* 67:948, 1965.
20. Menkes, J.H.: Read before the Conference on Treatment of Phenylketonuria, Cincinnati, Ohio, June 1966.
21. Wooley, D.W., and Van Der Hoeven, T.: Serotonin Deficiency in Infancy as One Cause of Mental Defect in Phenylketonuria, *Science* 144:883, 1964.