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Phenylketonuria VIII. Relation between Age, Serum Phenylalanine Level, and Phenylpyruvic Acid Excretion.* (22880)

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In phenylketonuria the liver enzyme system which oxidizes phenylalanine to tyrosine is defective(1); and phenylalanine accumulates in the blood(2). Phenylalanine(3) itself, and its metabolites, phenylpyruvic acid (3), phenyllactic acid(3) and phenylacetylglutamine(4) are excreted. Previous attempts to relate blood or urine levels of phenylalanine(2,5,6) or phenylpyruvic acid(6,7) to the development of the mental deficiency which accompanies the biochemical defect have not shown a significant correlation, nor have blood levels of phenylalanine appeared to correlate well with phenylpyruvic acid excretion. In the course of recent work in this laboratory it became apparent that there is a rough correlation between the level of phenylalanine in the fasting serum of patients and their excretion of phenylpyruvic acid. Also, children under the age of 3 years generally have been found to have much higher blood levels of phenylalanine than have older patients, in most of whom a rather consistent level (30 to 50 mg/100 ml) is observed.

Methods. Serum phenylalanine determinations were made on samples collected in the morning before ingestion of food; this usually has allowed a period of 15 hours for clearance of excess dietary phenylalanine from blood. Modification of the method of Kapeller-Adler (8) described previously(9) has been used for these determinations; phenylacetylglutamine and β-phenyllactic acid do not interfere. Either the method of preparation of the TCA filtrate or determination itself may have led to higher values than have been reported recently(10). Our data for normal sera are in agreement, however, with those obtained by other workers(11), and, in any event, should be internally consistent, since all determinations were carried out in the same manner. It should be noted that the method of Kapeller-Adler is inherently inaccurate when sera containing low levels of phenylalanine are assayed; at the higher levels observed with most

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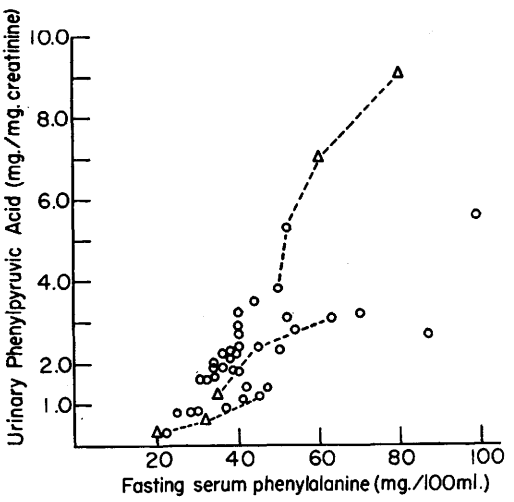

FIG. 1. Phenylpyruvic acid excretion and fasting serum phenylalanine levels of patients with phenylketonuria. △, values obtained with patients on synthetic diet deficient in or supplemented with phenylalanine. Dotted lines connect different values obtained with the same individual.

phenylketonuric patients it is sufficiently accurate to justify the conclusions made from the data.

Phenylpyruvic acid (PPA) has been determined by the following modification of the method of Penrose and Quastel(12): approximately 10 ml of urine is acidified to Congo Red by the addition of conc. HCl, stirred with 0.5 g of Lloyd's reagent, and filtered. 2 ml of filtrate (or suitable dilution) is placed in 50 ml volumetric flask, 2 ml of dinitrophenylhydrazine reagent (1.4 g dinitrophenylhydrazine in 500 ml 1 *N* HCl, warmed to dissolve, cooled, and stored in dark bottle) is added, and the mixture is allowed to stand 15 minutes at room temperature. 8 ml of 1 *N* NaOH is then added, the solution is diluted to 50 ml, filtered into a cuvette, and the optical density is measured immediately at 520 mμ. A standard curve is prepared with solutions containing from 0.01 to 0.20 mg PPA per ml; with a Coleman Junior Spectrophotometer and with cuvettes having a diameter of 22 mm these give a transmittance ranging from 88 to 11%. Determinations of PPA have been made on spot collections of urine and related to the creatinine content of the sample. The samples examined have ranged from consecutive 24 hour collections on the same patient to small samples such as those obtained from infants. The values shown in Table I represent concurrent determinations of serum phenylalanine and PPA. Values for PPA excretion obtained on different spot samples from the same patient have been quite consistent.

Results. Phenylpyruvic acid excretion. The data shown in Fig. 1 indicate a rough correlation between fasting serum phenylalanine level and amount of PPA excreted. However, a considerable variation exists in rate of PPA excretion by different individuals having approximately the same level of serum phenylalanine. Values obtained with different samples of urine from the same individual were consistent, however, and usually did not vary more than ± 0.3 mg PPA/mg creatinine. It is probable that the differences between individuals with the same serum phenylalanine level are influenced by activities of enzyme systems rather than because of differences in their intake of phenylalanine. This is indicated by data from 3 patients for whom PPA excretion was measured at widely varying phenylalanine levels; the values obtained with these patients are connected with the broken lines on Fig. 1. The younger children who might be used to confirm this by following a change in serum phenylalanine and in PPA excretion with increasing age have been maintained on a phenylalanine-restricted diet (9); it has therefore not been possible to obtain more data. The therapeutic value of phenylalanine-restricted diets for phenylketonuric infants of necessity will hamper future biochemical studies of phenylketonuria in younger children. The data shown in Fig. 1 serve to emphasize another point previously noted(9): namely, PPA is no longer detectable in phenylketonuric urine when the serum phenylalanine level falls below 15 to 20 mg %. Many samples of urine obtained from patients having serum phenylalanine levels of about 15 mg % have been examined with both the ferric chloride and dinitrophenylhydrazine tests and also chromatographically (13) for PPA with negative results. This is of significance in two regards: First, in assessing the status of patients receiving phenyl-

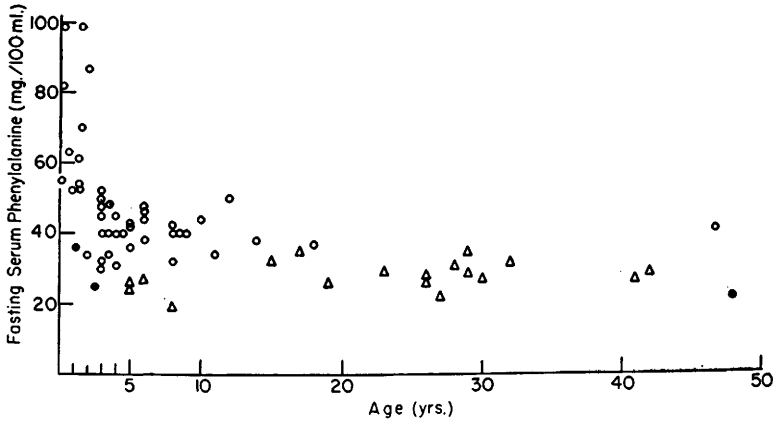

FIG. 2. Age and fasting serum phenylalanine levels of patients with phenylketonuria. ●, atypical cases commented upon in text. △, values taken from Borek, *et al.*(5).

alanine-restricted diets, a negative test for phenylpyruvic acid does not assure that an adequately low blood level of phenylalanine has been obtained, although a faint positive reaction certainly indicates 15 mg % or higher. Second, it is entirely possible that persons may exist having the trait of phenylketonuria, but with an incomplete block in the oxidation of phenylalanine so that they have higher than normal levels of phenylalanine but do not excrete phenylpyruvic acid; a case was reported recently(14) who almost belongs in this category. The only manner in which such an individual would be detected would be by a serum phenylalanine determination, or by chromatographic examination of his urine for the abnormal metabolites, *o*-hydroxyphenylacetic, *p*-hydroxyphenylacetic, and indolelactic acids.

Fasting serum phenylalanine levels. In Fig. 2 the values for serum phenylalanine in patients are plotted against the age of the patients. Very few adult phenylketonurics have been encountered in the work reported here; for values from adult patients, the data reported by Borek, *et al.*(5) have been included. They used essentially the same analytical procedure so their values should be consistent with ours. It can be seen that fairly consistent values, ranging between 20 and 50 mg% are found with patients older than 3 years. Patients younger than 3 years, however, usually show very much higher levels of phenylalanine; two children having fasting serum levels as high as 99 mg% have been observed.

Because of the considerably elevated blood phenylalanine levels, the urinary excretion of phenylpyruvic acid would be increased, as shown above. It would also follow that blood levels of phenylpyruvic acid and an increased degree of other biochemical abnormalities should occur in young children, and as a consequence, physiological and clinical symptoms should be accentuated at this age. This idea is supported by observations that electroencephalographic abnormalities are more severe in younger patients(15,16), and that abnormal liver function as manifested by the presence of abnormal β-globulins(17) or abnormal galactose tolerance curves(18) is observed more frequently in this group. In addition, observations which will be reported elsewhere have been strongly suggestive that severe damage to the nervous system probably occurs only in children under the age of 2 to 3 years.†

In regard to a possible relation between the degree of biochemical abnormalities and the severity of mental impairment in patients, it is interesting to note that the patients shown as closed circles on Fig. 2, who show lower values for serum phenylalanine than do other patients of the same age, are individuals with atypically high mental ability; two of them have been discussed elsewhere(13).

Incidence and age distribution of phenyl-

† Unpublished observations.

ketonuria in Utah. Few adult patients with phenylketonuria have been located in Utah. So far, 18 phenylketonurics born in Utah have been found; 11 of these are at present institutionalized. One is a 50-year-old man, one a 47-year-old woman, one a 20-year-old man, and the remainder are all 16 years or younger. Earlier studies in other localities have given no indication of a decrease in the incidence of phenylketonuria in older mental patients; indeed, the good physical health of most patients has been noted(19). It seems improbable that a considerable number of older patients with phenylketonuria have escaped institutionalization in Utah, hence some other reason for the increased proportion of younger patients must be sought. It seems reasonable to propose that the recent advent of sulfa drugs and antibiotics has increased the survival rate of affected children, many of whom are particularly susceptible to respiratory infections. If this is true, the older estimates of the incidence of phenylketonuria in the eastern United States of about 1 in 26,000(20) might need revision.

In Utah, there were recorded 224,576 live births from January 1, 1946 to December 31, 1955. Eleven of the patients reported in Table I were born in Utah during this 10-year period, which would give an incidence of 1 in 20,400. Since it is almost certain that all phenylketonuric children born in this period have not been detected, the present incidence of phenylketonuria in children being born in Utah must be somewhat greater than 1 in 20,000. An alternative proposal, that a different racial stock in the area might have led to this increased incidence, is controverted by the comparatively small number of older patients.

Summary. 1. The excretion of phenylpyruvic acid by patients with phenylketonuria is roughly proportional to their fasting serum phenylalanine levels. Phenylpyruvic acid is not detectable in urine of patients with serum levels below 15 mg% phenylalanine. 2. Children less than 3 years old frequently show much higher serum levels of phenylalanine than older patients. 3. The incidence of known phenylketonuria in Utah during the

TABLE I. Urinary Phenylpyruvic Acid, Fasting Serum Phenylalanine, and Age of Patients with Phenylketonuria.

Name	Age (yr)	Fasting serum phenylalanine (mg/100 ml)	Urinary phenylpyruvic acid (mg PPA per mg creatinine)
James A.†	11	34	1.9
Linda B.†	9	40	2.7
Brent C.	13 mo	54	
Robert C.	3	48	
Florence Da.†	4	45	1.2
	"	32*	.6*
	"	20*	.3*
Judy Da.†	6	46	
	8	40	2.9
Dean D.†	17		1.1
	18	37	.9
Patricia D.†	10	44	
	12	50	2.3
Allan Du.†	4	40	
	5	36	2.2
	6	38	2.3
Jill Du.†	13 mo	36	1.9
	2	34	2.0
	3	40	2.2
	5	41	1.8
Robert Du.†	7 mo	63	3.1
	8 "	17*	not det.‡
	9 "	35*	1.2*
	3	45	2.4
Mary D.	8	42	
Mary J.	10 mo	52	5.3
	10 "	10*	not det.‡
	16 "	60*	7.0*
	17 "	80*	9.1*
	3	50	3.8
John J.	4	40	2.4
David J.	3	52	3.1
Debra L.†	2½	25	.8
Anne La.†	6	44	3.5
Gary La.†	8	40	1.8
Sharon L.	3	32	1.6
Ruth L.†	47	41	1.1
Sheila M.	17 mo	54	2.8
Darryle Ma.	2 "	55	
Wauneta Ma.	4	31	1.6
Cheryl M.†	15 mo	61	
Kai N.	3	30	.8
Stephen O.	2½ mo	82	
Dennis Pr.	5	42	1.4
Norris Pr.	2	87	2.7
David R.†	13		2.5
	14	38	2.3
John S.	8	32	
Wilfred S.†	3½	34	1.7
Mary T.†	6	47	1.4
Kathy T.	18 mo	99	5.6
Clifford W.†	48	22	.3
	49		.3
Debra W.	18 mo	70	3.2
Mark Y.	4 "	99	

* Values obtained while patients were receiving synthetic diets, either deficient in or supplemented with phenylalanine. † Patients born in Utah. ‡ not det. = not detectable.

past 10 years has been 1 in 20,400 births.

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Direct Complement Fixation by Turkey and Chicken Serum in Viral Systems.*† (22881)

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Bushnell and Hudson(1) used both complement fixation and agglutination procedures for diagnosis of *Salmonella pullorum* in chicken and turkey serum. They were able to get fixation of complement with unheated serum only. The failure to produce reactions with inactivated serum was attributed to destruction of the antibody by heat. In addition they stated that concentration of serum greater than 1/50 could not be read because of the anti-complementary quality of the serum. Rice(2) repeated this work and encountered essentially the same difficulties. She did not attribute the inability of heated serum to fix complement to destruction of antibody, but rather to the presence of inhibitory or compensatory factor in certain fowl sera. The result of this work was the development of the indirect complement fixation method(3).

In the following procedure unheated turkey and chicken sera are used in direct complement fixation. The test is qualitative in nature, designed for diagnostic work where large numbers of specimens are involved. Three viral systems have been tested, ornithosis, Newcastle disease and infectious bronchitis.

Materials and methods. The methods for direct and indirect complement fixation were essentially those of Hilleman, Haig and Helmold(4), *i.e.* all reagents were adjusted to 0.2

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