PHENYLPYRUVIC OLIGOPHRENIA. REPORT OF A CASE IN AN INFANT WITH QUANTITATIVE CHEMICAL STUDIES OF THE URINE

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The object of this study is to present quantitative determinations of phenylalanine, tyrosine, and some of their derivatives, excreted by a two-year-old boy with clinical and urinary findings diagnostic of the rare condition known as phenylpyruvic oligophrenia. This metabolic aberration, manifested by the excretion of phenylpyruvic acid, was first described by Fölling in 1934 (1) in 10 feeble-minded patients, and cases have subsequently been reported by Penrose (2), Jervis (3, 4), and others. The disease is characterized clinically by marked mental deficiency, frequently accompanied by athetosis and other neurological stigmata. Many of the patients are well-developed physically, and even attractive in appearance. Blond hair and complexion and a susceptibility to eczema are frequently observed. The incidence among institutionalized feeble-minded individuals of all ages is about 0.8 per cent. On the basis of extensive genetic studies, Jervis (4) confirmed Penrose's opinion (5) that the anomaly is determined by a single autosomal recessive gene.

The quantity of phenylpyruvic acid in the urine varies with the protein intake, but even on a protein-free diet some is always excreted, derived probably from body protein. The excretion of the compound is greatly increased by the administration of phenylalanine, protein (casein), or phenylpyruvic acid, and to a lesser extent by phenyllactic acid, whereas other amino acids, including tyrosine, have no effect on its output. Thus the defect is, apparently, limited to the metabolism of phenylalanine.

REPORT OF CASE ¹

S. W., a boy aged 25 months, was admitted on November 25, 1941, with the chief complaints of retardation of development and eczema, after a urine specimen had given a positive qualitative test for phenylpyruvic acid.

¹ We are grateful to Dr. T. D. Van Orden for the privilege of observing this patient.

The family history was positive for allergic manifestations, including hives in the father, and eczema and asthma in a brother. There was no family history of nervous illness. The only sibling, a brother, had been slow in development, having walked at 2 years of age and talked at 3, but was described by his pediatrician as entirely normal at his present age of 4. Tests of the father's, mother's, and brother's urines for phenylpyruvic acid were negative. The pregnancy was complicated by severe nausea and vomiting, and by a small pelvis which necessitated delivery by Caesarian section. The patient had been subject to a severe eczematous eruption since the age of 2 months. Mental retardation had been suspected at 4 months. The infant failed to raise his head until the age of one year, and began to take notice of his surroundings and to recognize his parents at 20 months. His first tooth erupted at 6 months. He sat without support for no more than a few minutes, and did not form words. He did not reach for objects but he seemed to take pleasure in holding a toy when it was placed in his hand.

Convulsions were never observed, but the infant was said to "jump" occasionally, with momentary flexion of the head and extension of all 4 extremities.

Physical examination revealed a large, obese, flabby-muscled, blond boy of 26 months, who could sit unsupported for a few minutes and could maintain himself on his arms and knees in a crouching position. Most of the time he lay quietly on his back. He followed a light or a large object with his eyes, and responded sluggishly to sounds. He had an attractive facies with a smiling expression. He was goodnatured and contented most of the time, but occasionally cried and sometimes scratched his face, arms, and legs which were the sites of a dry, scaly, patchy, eczematous eruption. The child weighed 14 kilograms, and the body measurement were as follows: head circumference 46 cm., chest 55.5 cm., abdomen 54 cm., stem length 56 cm., total length 89 cm. The head appeared somewhat small in proportion to the body, but was well-formed. The fontanelles were closed. The eyes were blue, the pupils not unusual and the fundi normal. Except for moderately hyperactive deep reflexes the neurological examination was negative, and the remainder of the physical examination revealed no abnormal findings.

Roentgenograms of the skull and wrists were within normal limits. The Kline, Schick, and Mantoux tests were negative, and routine urinalyses and blood count were

normal. The blood plasma ascorbic acid level which on admission was 0.7 mgm. per cent fell to 0.3 mgm. per cent after 9 days of low ascorbic acid intake, and rose to 0.6 mgm. per cent within 4 hours after an oral test dose of 200 mgm. of ascorbic acid.

Psychometric examination by a consultant psychologist confirmed the clinical impression of marked mental retardation.

On admission, the child was given a measured diet, low in ascorbic acid but adequate in thiamin and other vitamins, and calculated to provide 4 grams of protein per kilogram of body weight, daily. The exact amount of nitrogen consumed, including that in added amino acids, is recorded in Table I. A description of the chemical methods and a report of the urinary findings follows.

CHEMICAL METHODS

The method described by Hoag (6) for the quantitative collection of urine in young male infants permitted the collection of 22 12-hour urine specimens without loss. Voidings were timed to the nearest minute, the actual collection periods varying usually between 11 and 13 hours. Results were calculated in terms of 12-hour periods. The specimens were preserved with toluene.

Total urinary nitrogen was determined by the macro-Kjeldahl method.

Phenylpyruvic acid may be detected by the development of a transient but characteristic green color upon the addition of 5 per cent ferric chloride to acidified urine. This reaction forms the basis of a quantitative method, worked out by Jervis, Block, Bolling, and Kanze (7) for blood and spinal fluid filtrates, and has been adapted for the determination of the compound in urine.

Phenylalanine was determined by a further modification of the Kapeller-Adler method (8) as recently modified by Block and Bolling (9). The urine was acidified and extracted with ether for 24 hours in a continuous extractor. This procedure removed phenylpyruvic and phenyllactic acids which interfere with the determination of phenylalanine. Tyrosine was removed by treatment with potassium permanganate in the cold, as recommended by Kapeller-Adler (8).

The optical isomers of phenylalanine were determined by means of the d-amino acid oxidase. The enzyme was extracted with M/60 sodium pyrophosphate solution at pH 8.0 from acetone-dried sheep kidneys, as described by Negelein and Brömel (10). Their more elaborate procedure ("step 1") was found unnecessary; correction for the error introduced by the small amount of acetone remaining in the kidney extract was made by a suitable blank. This enzyme converts the d-portion of d,l-phenylalanine into the corresponding keto acid (phenylpyruvic). The urine to be tested was extracted with ether to remove phenylpyruvic acid and then incubated with the enzyme for 3 hours at 37° C. Proteins were precipitated with trichloroacetic acid, and phenylpyruvic acid determined in the filtrate by the dinitrophenylhydrazone method of Penrose and Quastel (11a), as modified by Sealock

(11b). From the amount of phenylpyruvic acid produced, could be calculated the d-phenylalanine in the urine, and l-phenylalanine could be calculated by difference.

Jervis and his co-workers (7) have reported that phenyllactic acid gives almost as much color in the nitration process of the Kapeller-Adler method as does phenylalanine. Consequently, the method was used for the determination of phenyllactic acid as follows: To the urine was added an equal volume of saturated sodium bisulfite solution. This formed an addition compound with phenylpyruvic acid which was insoluble in ether. The urine-bisulfite mixture was then made just acid to Congo red with sulfuric acid and extracted with ether for 24 hours in a continuous extractor. The ether extract, containing phenyllactic acid, but no phenylalanine or phenylpyruvic acid, was then used for the determination of the former acid by the Kapeller-Adler method. For the purposes of this study, it appears reasonable to assume that the compound determined was actually phenyllactic acid, but complete verification must await isolation of the pure compound from the urine. If error exists, it is presumably in the direction of making the reported amounts of phenyllactic acid too high.

The Millon method as modified by Folin and Ciocalteu (12) was applied to the urine both before and after extraction with ether. Thus the total hydroxyphenyl compounds were divided into ether-soluble and ether-insoluble components.

RESULTS

Daily excretion of metabolic products

The results, calculated on the basis of 12-hour periods, are presented in Table I.

Phenylpyruvic acid. The patient always excreted phenylpyruvic acid in amounts varying from 0.45 to 1.03 grams per 24 hours. The gradual rise in excretion during the period of observation may perhaps have been due to his higher than customary intake of protein. In adult patients on a normal diet, Jervis (13) reported a daily output of 1.8 to 2.1 grams of phenylpyruvic acid, and Penrose and Quastel (11a), 1.4 grams.

Phenyllactic acid. The daily output of phenyllactic acid varied from 0.29 to 0.55 gram per 24 hours. Comparable figures for adult patients are not available.

Phenylalanine. The daily urinary output of phenylalanine varied from 0.21 to 0.32 gram. Because a search of the literature revealed no information on the normal urinary excretion of phenylalanine, a series of 11 24-hour urine specimens, from 5 children of comparable age and receiving similar diets, was analyzed. The results are presented in Table II, together with values

TABLE I
Urinary excretion of protein derivatives in phenylpyruvic oligophrenia

Date	Weight	Nitrogen		Phenylalanine derivatives					Tyrosine derivatives		Substance ingested
		Intake	Urinary output	Phenyl-pyruvic acid	Phenyl-lactic acid	Phenylalanine			Ether-soluble expressed as tyrosine	Ether-insoluble expressed as tyrosine	
						Total	d-form	l-form			
	kgm.	mgm. per kgm. per 24 hours	mgm. per kgm. per 18 hours	mgm. per 18 hours					mgm. per 18 hours		grams
November 27 to 28	14.3		175 200	258 195	134 159	101 107	4 11	97 96	28 27	15 10	
November 28 to 29	14.6	672	200 180	260 271	245 265	124 139	19 5	105 134	28 47	20 14	
November 29 to 30	14.6	579	160 188	332 328	273 233	157 146	26 20	131 126	94 120	28 30	7.3 l-tyrosine
November 30 to December 1	15.0	640	200 250	454 461	276 273	168 155	29 23	139 132	78 105	38 57	
December 1 to 2	14.8	730	220 191	1988 1332	566 910	1951 686	1619 323	332 363	118 63	51 33	7.4 d,l-phenylalanine
December 2 to 3	14.8	640	274 219	1294 737	366 437	367 200	66 32	301 168	77 56	38 17	
December 3 to 4	14.8	592									
December 4 to 5	14.7	656	247 188	517 512	263 291	153 159	39 30	114 129	56 44	20 15	
December 5 to 6	14.6	656	209 142	508 356	233 152	179 91	35 24	144 67	52 32	17 11	0.2 ascorbic acid 0.2 ascorbic acid
December 6 to 7	14.9	640	193 198	446 447	199 200	163 141	18 13	145 128	37 34	13 14	0.2 ascorbic acid 0.2 ascorbic acid
December 7 to 8	14.8	683	184 205	2141 1997	595 470	1388 362	844 156	544 206	54 53	14 16	0.2 ascorbic acid 7.45 d,l-phenylalanine 0.2 ascorbic acid
December 8 to 9	14.8	736	222 212	1069 666	327 240	261 190	35 9	226 181	19 25	53 32	0.2 ascorbic acid

obtained in previous studies in infants. In no instance did the phenylalanine excretion exceed 53 mgm. per 24 hours (4.52 mgm. phenylalanine nitrogen), the average being 30 mgm. per 24 hours (2.5 mgm. nitrogen) which is less than one-seventh the average amount excreted by our patient. In the normal children, the phenylalanine nitrogen amounted to 0.073 per cent of the total urine nitrogen whereas, in the patient with phenylpyruvic oligophrenia, the phenylalanine nitrogen represented 0.42 per cent of the total nitrogen. From this, it would appear that this patient, in contrast to the patient studied by Jervis (13), excreted phenylalanine in the urine in amounts significantly above normal.

The larger part of excreted phenylalanine was of the natural l-form. From 4 to 26 per cent (average 14 per cent) was d-phenylalanine. If this figure is to be considered valid in view of the technical difficulties inherent in the determinations of small amounts of d-phenylalanine, it must be

explained on the basis of racemization in the body, inasmuch as phenylalanine in the food is undoubtedly l-phenylalanine. Such *in vivo* racemization has been shown to occur in the case of d-phenylaminobutyric acid (du Vigneaud and Irish (14)). Racemization would be consistent with the theory of Jervis and his colleagues (7) that the excess phenylalanine in the blood of phenylpyruvic oligophrenics is formed from phenylpyruvic acid, although Fölling, Closs, and Gammes (15) maintained that the excess phenylalanine found in the blood in their cases was all the levo form.

Tyrosine derivatives. The daily excretion of hydroxyphenyl compounds averaged 77 mgm. for the ether-soluble and 30 mgm. for the ether-insoluble portion. In the 5 normal subjects concerning whom other data are presented in Table II, an average figure for the ether-insoluble fraction was 27 mgm. There is, therefore, no evidence of any abnormal excretion of hydroxyphenyl compounds in this case of phenylpyruvic oligophrenia.

TABLE II

Urinary excretion of phenylalanine and tyrosine by young infants and children compared to that of the patient with phenylpyruvic oligophrenia, per 24 hours

Subject	Age	Weight	Protein intake	Total nitrogen in urine	Tyrosine* nitrogen	Phenylalanine† nitrogen	Phenylalanine as per cent of total nitrogen
		kgm.	grams per kgm.	grams	mgm.	mgm.	
D. S. Eczema	3½ years	13.0	6.8	8.43 6.68	3.48 2.89	4.52 3.28	0.054 0.049
P. M. Tuberculosis	2½ years	14.2	2.6	1.62 2.32 1.69	2.25 2.21 2.20	2.23 1.78 3.36	0.138 (Thymol)§ 0.077 0.199 (Thymol)
J. L. Tuberculosis, malnutrition	4 years	13.4	3.5 4.7	3.53 5.38	1.31 2.50	2.09 4.41	0.059 0.082
A. K. Dwarfism	3½ years	8.7	4.1 2.9	2.32 4.47	0.60 1.41	0.66 1.68	0.028 0.038
W. K. Mastoid, post-operative	5 years	17.5	4.4 4.0	5.77 4.71	2.34 1.75	2.31 1.64	0.040 0.035
Premature infants 24 days on 9 infants	8 to 25 days	1.82 to 3.14	6.0	0.96	1.93	2.25	0.230
Full term infants 3 days on 2 infants	17 to 21 days	3.53 to 3.88	4.5	1.66	0.88	2.55	0.150
S. W. Phenylpyruvic oligophrenia	2 years	14.5	4.2 4.1	5.68 5.78	4.10 2.32	23.29 25.06	0.41 0.43

* One mgm. tyrosine nitrogen is equivalent to 12.93 mgm. of the amino acid.

† One mgm. phenylalanine nitrogen is equivalent to 11.79 mgm. of the amino acid.

§ Poor specimen—several enureses.

Effect of ingestion of tyrosine

Seven and three-tenths grams l-tyrosine (0.5 gram per kgm.) was administered by mouth in a single dose without significant effect on the urinary excretion of phenylpyruvic acid or phenylalanine (Table I). The slight increase in tyrosine and its derivatives represented roughly 2 per cent of the amount ingested and is not indicative of any fault in the metabolism of tyrosine. This confirms the results obtained by Jervis (13).

Effect of ingestion of phenylalanine

The ingestion of 7.4 grams d,l-phenylalanine (0.5 gram per kgm.) caused a marked increase in the urinary excretion of phenylpyruvic and phenyllactic acids and phenylalanine. In the first 24 hours, 2.404 grams phenylpyruvic acid, 0.926 gram phenyllactic acid, and 2.313 grams phenylalanine were excreted in excess of the foreperiod levels, thus accounting for approximately 76 per cent of

the ingested dose (Table III). Within 48 hours, the extra excretion had amounted to 98 per cent of the ingested dose.²

The maximum excretion of phenylpyruvic acid and phenylalanine was reached during the first 12 hours, whereas phenyllactic acid excretion reached its peak during the second 12 hours.

The magnitude of phenylpyruvic acid increase after phenylalanine is comparable to that observed by Penrose and Quastel (11a) who gave 3 grams of phenylalanine to phenylpyruvic oligophrenic adults and obtained an excretion of extra phenylpyruvic acid equivalent to the total phenylalanine fed.

² As was suggested in the description of methods, the values for phenyllactic acid may be too high, both in foreperiods and after amino acid ingestion. Even disregarding entirely this fraction, a minimum of 64 per cent of ingested phenylalanine is accounted for in the first 24 hours by the excretion of phenylalanine and phenylpyruvic acid, and 82 per cent in 48 hours.

TABLE III

Urinary excretion of phenylalanine derivatives after ingestion of d,l-phenylalanine

	Phenyl- pyruvic acid	Phenyl- lactic acid	Phenylalanine		Total
			d-form	l-form	
INGESTION OF 7.4 GRAMS d,l-PHENYLALANINE					
Foreperiod level—mgm.	458	275	26	136	
Extra excretion					
First 12 hours—mgm.	1530	291	1593	196	
Per cent of intake*	20.7	3.9	21.5	2.6	48.7
2nd 12 hours—mgm.	874	635	297	227	
Per cent of intake	11.8	8.7	4.0	3.1	27.6
3rd 12 hours—mgm.	836	91	40	165	
Per cent of intake	11.3	1.2	0.5	2.2	15.2
4th 12 hours—mgm.	279	162	6	32	
Per cent of intake	3.8	2.2	0.1	0.4	6.5
					98.0
INGESTION OF 7.45 GRAMS d,l-PHENYLALANINE†					
Foreperiod level—mgm.	447	200	16	137	
Extra excretion					
First 12 hours—mgm.	1694	395	828	407	
Per cent of intake	22.7	5.3	11.1	5.5	44.6
2nd 12 hours—mgm.	1550	270	140	69	
Per cent of intake	20.8	3.6	1.9	0.9	27.2
3rd 12 hours—mgm.	622	127	19	89	
Per cent of intake	8.3	1.7	0.3	1.2	11.5
4th 12 hours—mgm.	219	40	—7	44	
Per cent of intake	2.9	0.5	—0.1	0.6	3.9
					87.2

* The molecular weights of these three compounds are so nearly identical that calculations of percentages or sums of percentages are the same whether reported "as phenylpyruvic acid," "as phenyllactic acid" or "as phenylalanine."

† Diet unchanged except for ingestion of 0.4 gram ascorbic acid daily.

The high excretion of phenylalanine, both before and particularly after the ingestion of this substance, was in contrast to the observation of Jervis (13) who found none at all in the urine of a phenylpyruvic oligophrenic, even after administering phenylalanine by mouth. Fölling, Closs, and Gamnes (15), using a qualitative method, reported that an increased excretion of phenylalanine followed the oral or intravenous administration of d-phenylalanine to their human subjects.

It is interesting to note that only about 6 per cent of the ingested d,l-phenylalanine was excreted as the l-isomer, whereas 13 to 25 per cent of it was excreted as d-phenylalanine. Presumably the body excreted more of the unnatural enantiomorph which is less readily metabolized.

Effect of ascorbic acid

Because vitamin C has been shown to prevent or relieve a defect in the metabolism of tyrosine and phenylalanine exhibited by premature infants (16), it was tested in this case of phenylpyruvic oligophrenia (Tables I and III). It was completely without effect in relieving the spontaneous phenylketonuria and did not prevent the increased excretion of the metabolic products of phenylalanine after the administration of the amino acid. It is unlikely that the small differences in the rate and pattern of excretion of the derivatives in the two sets of observations following phenylalanine ingestion are attributable to ingested ascorbic acid. Jervis (17) found similar results in his patients.

DISCUSSION

The path of normal metabolism of phenylalanine is still a matter of speculation. Presumably, it is first deaminized to form the -keto acid, phenylpyruvic acid, which may then be completely oxidized. There is, however, evidence that at least some phenylalanine may be converted in the body to tyrosine or its metabolic products, p-hydroxyphenylpyruvic and p-hydroxyphenyllactic acids (18). In phenylpyruvic oligophrenia, there is apparently no impairment in the metabolism of tyrosine. Even after the administration of tyrosine or phenylalanine there is no significant increase in urinary excretion of substances giving the Millon reaction. This finding, taken in conjunction with the fact that ingested phenylalanine can be accounted for almost quantitatively as excess phenylpyruvic and phenyllactic acids and phenylalanine, would indicate that phenylpyruvic oligophrenics do not metabolize phenylalanine by way of tyrosine. It is interesting to speculate whether tyrosine, which is not considered an essential amino acid in normal individuals, may be essential in these cases. If patients with phenylpyruvic oligophrenia are unable to form tyrosine from phenylalanine or its metabolic products, then

they must be dependent on a dietary intake of tyrosine for their metabolic needs of this amino acid.

Undoubtedly, phenylalanine is deaminized to phenylpyruvic acid, but the mechanism and site of this deamination are still uncertain. If it takes place chiefly in the liver, one would expect to find phenylpyruvic acid in the blood in the process of transportation to the kidneys. Both Fölling, Closs, and Gamnes (15) and Jervis and his colleagues (7) were unable to find significant amounts of phenylpyruvic acid in the blood of their patients, but they did find abnormally large amounts of phenylalanine in the blood. Jervis concluded that the essential defect of the subjects is an inability to dispose of phenylalanine at a normal rate rather than a failure to break down phenylpyruvic acid, as had been previously assumed. The presence of phenylpyruvic acid in the urine he considered to be an incidental phenomenon, resulting from the deamination of a portion of the blood phenylalanine by kidney tissue.

The excretion by our subject, S. W., of large amounts of phenylalanine after ingestion of this substance may have been due to the size of the dose given (7.4 grams, 0.5 gram per kgm. of body weight), which was much greater than that which Jervis (13) administered to his patient. The amounts of phenylalanine in the urine of S. W. were of the same order of magnitude as those found in normal infants given comparable doses (18). The latter, however, never exhibited the most striking metabolic characteristic of the phenylpyruvic oligophrenic, namely, the excretion of significant amounts of phenylpyruvic acid, and they did, in the absence of vitamin C, show evidence of conversion of phenylalanine to tyrosine which was not observed in the oligophrenic subject.

The clinical value of the qualitative test for phenylpyruvic acid in the urine of infants suspected of mental retardation should be emphasized. The test should be applied especially in those retarded children who are blond in coloring, and whose mental deficiency is suspected on the basis of slowness in acquiring behavior patterns and yet seems to be belied by an attractive facial appearance. The presence of eczema, of a family his-

tory positive for mental deficiency, or of neurological manifestations characteristic of extrapyramidal tract involvement, should also lead to a suspicion of this disease, though their absence does not rule it out.

SUMMARY

1. Studies were carried out on the metabolism of phenylalanine and tyrosine in a patient with phenylpyruvic oligophrenia.

2. The patient always excreted phenylpyruvic acid in the urine in amounts varying from 0.45 to 1.03 grams per 24 hours. The daily excretion of phenyllactic acid varied from 0.29 to 0.55 gram and of phenylalanine from 0.21 to 0.32 gram per 24 hours.

3. The administration of d,l-phenylalanine (0.5 gram per kgm.) resulted in a prompt rise in the excretion of phenylpyruvic and phenyllactic acids and phenylalanine. Within 24 hours after feeding, 76 per cent of it had been excreted; 32 per cent as extra phenylpyruvic acid, 13 per cent as phenyllactic acid, and 31 per cent as extra phenylalanine, about three quarters of the latter being d-phenylalanine and the remainder the l-isomer. Within 48 hours, the extra excretion had amounted to 98 per cent of the ingested dose.

4. The administration of l-tyrosine in similar dosage (0.5 gram per kgm.) had no significant effect on the excretion of phenylpyruvic acid, nor was there any evidence of an abnormality in the metabolism of tyrosine.

5. The administration of large doses of ascorbic acid (0.4 gram per day) had no effect, either on the spontaneous excretion of phenylpyruvic acid or on the increased excretion of metabolic products following the ingestion of phenylalanine.

6. The absence in the urine of significant amounts of hydroxyphenyl compounds in this patient, spontaneously and even after the ingestion of phenylalanine, suggests that the conversion *in vivo* of phenylalanine to tyrosine, previously shown to take place in the normal organism, is deficient in patients with phenylpyruvic oligophrenia. This finding is consistent with the thesis advanced by Jervis and his coworkers (7) that in this disease the primary metabolic defect is an inability to handle phenylalanine.

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