

Tyrosyluria in marasmus

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1. Plasma tyrosine and urinary *p*-hydroxyphenyl lactic acid (PHPLA) and *p*-hydroxyphenyl acetic acid (PHPAA) were studied in thirty patients with marasmus and twenty normal controls in the same age group.

2. In the control group conventional tyrosyluria was not observed but 30% of the group excreted high levels of PHPAA. In the group with marasmus, plasma tyrosine and urinary PHPLA and PHPAA values were significantly higher than the control values. However only 13.3% of the patients were considered to have conventional tyrosyluria and 52.3% were found to excrete high levels of PHPAA.

3. Administration of ascorbic acid resulted in a reduction of PHPLA excretion while it had no effect on PHPAA excretion.

4. It was inferred that (a) tyrosyluria in marasmus is due to the reduced activity of the hepatic enzyme 4-hydroxyphenyl pyruvate: oxygen oxidoreductase (hydroxylating, decarboxylating) (PHPPA-oxidase; EC 1.13.11.27) due to the deficiency of ascorbic acid and (b) high excretion of PHPAA is related to age and nutrition of the child and is unaffected by the administration of ascorbic acid.

5. It was further inferred that urinary excretion of PHPLA is a reliable index of tyrosyluria.

Increased levels of plasma tyrosine and increased urinary excretion of its metabolites *p*-hydroxyphenyl pyruvic acid (PHPPA), *p*-hydroxyphenyl lactic acid (PHPLA) and *p*-hydroxyphenyl acetic acid (PHPAA) associated with liver damage are due to the reduction of hepatic enzymes 4-hydroxyphenol pyruvate: oxygen oxidoreductase (hydroxylating, decarboxylating) (PHPPA-oxidase; EC 1.13.11.27) and glutamate tyrosine transaminase (EC 2.6.1.5) (Kirberger, 1954; Levine & Conn, 1967). Excess tyrosine inhibits the activity of PHPPA-oxidase and small amounts of ascorbic acid are needed to maintain the activity of the enzyme (Knox & Goswami, 1960). Thus tyrosyluria is also observed in scurvy (Morris *et al.* 1950). A transient neonatal tyrosyluria seen particularly in preterm babies results from a combination of factors which include delayed maturation of hepatic enzymes, inadequate intake of ascorbic acid, high protein intake (Levine *et al.* 1941; Menkes & Avery, 1963; Mathews & Partington, 1964). Dean & Whitehead (1963) observed tyrosyluria in kwashiorkor which they attributed to the deficiency of tyrosine metabolizing hepatic enzymes, indicating liver dysfunction. The problem has, however, not been investigated in marasmus.

MATERIALS AND METHODS

The subjects were thirty patients with marasmus weighing less than 60% of the 50th percentile of the Boston Standards (Vaughan III, 1975) and without oedema in the age group 6 months–3 years (mean age 20.6 months) admitted to the Paediatric Medical Wards of Medical College Hospital, Rohtak; and twenty normal controls in the same age group (mean age 19.8 months). On admission investigations included urine, stool and haematological investigations, serum protein estimation, tuberculin test and radiological examination of the patients, where indicated. Samples of heparinized blood and urine were obtained in both the marasmus and control groups after an overnight fast of 6–8 h. Plasma tyrosine was estimated by the procedure of Sidney & Cooper (1952). Phenolic acids were extracted from urine (Smith, 1969) and the extract corresponding to 0.6 ml urine was spotted on a

Table 1. Plasma tyrosine (mmol/l) and urinary *p*-hydroxyphenyl lactic acid (PHPLA) and *p*-hydroxyphenyl acetic acid (PHPAA) ($\mu\text{g/ml}$) in subjects with marasmus and control subjects aged 6 months–3 years

(Mean values and standard deviations)

Subjects	No. of subjects	Plasma tyrosine		PHPAA		PHPLA	
		Mean	SD	Mean	SD	Mean	SD
Control	20	0.06	0.01	7.91	5.5	(3.0 and 0.5)*	
Marasmus	30	0.10	0.05	21.1	18.5	5.08	6.01
Statistical significance of difference between groups		$P < 0.01$		$P < 0.001$			

* Detected in two controls and twenty-seven marasmic patients.

Whatman No. 1 paper and chromatographed for phenolic acids bidimensionally using solvent systems isopropanol-*n*-butanol-*ter*-butanol-ammonia-water (4:2:2:1:2 v/v) and ether-xylene-formic acid-water (50:30:10:3, v/v) for 12–15 h and 2.5 h respectively (Saini, 1963). Dried chromatograms were lightly sprayed with freshly prepared *p*-nitraniline (PNA) reagent (Smith, 1969). PHPLA and PHPAA were quantitated according to the method of Saini (1967). The spots were cut out and an equally sized paper in between the spots was also cut out to serve as blank. The paper pieces were placed in stoppered glass tubes and 0.4 ml of freshly prepared PNA reagent at 4° was added to drench the paper thoroughly. Colour was then extracted in 8 ml of 50% acetone-free aqueous methanol. After 2 h the supernatant was removed and centrifuged and optical density recorded at 540 m μ wave length against the blank. The quantitation was done with respect to standards processed similarly. The marasmic patients were then divided into two groups: one group (mean age 20.8 months) was given a diet containing 5.0 g milk protein and calcium caseinate/kg body-weight for 7 d and the second group (mean age 20.2 months) was given the same diet with the addition of 250 mg ascorbic acid/d for 7 d. At the end of the 7 d period plasma tyrosine and urinary PHPLA and PHPAA levels were estimated for both groups.

RESULTS

Plasma tyrosine and urinary PHPLA and PHPAA levels in the marasmus and control groups are recorded in Table 1. Values for these measurements revealed a significant rise in patients with marasmus compared to the controls. Furthermore PHPLA was detected in two of the twenty normal controls and in twenty-seven of the thirty patients. There was no case of tyrosyluria (PHPLA and PHPAA being less than 10 $\mu\text{g/ml}$) in the control group, but six subjects (30%) showed high PHPAA excretion (more than 10 $\mu\text{g/ml}$). On the other hand in the marasmus group there were four cases of conventional tyrosyluria (13.3%) and sixteen subjects (53.3%) showed high PHPAA excretion. The remaining ten patients with marasmus had low urinary levels of PHPLA and PHPAA. Except for one patient with tyrosyluria whose plasma tyrosine concentration was 0.28 mmol/l, the other patients, including the other three with tyrosyluria, had either normal or slightly raised plasma tyrosine levels.

Plasma tyrosine and urinary PHPLA and PHPAA values in marasmic patients after dietary treatment are recorded in Tables 2 and 3 and compared with the initial values. In the group of patients given the high-protein diet there was no significant change in any of these values. On the other hand marasmic patients given supplementary ascorbic acid showed a significant reduction in PHPLA excretion while the reduction in plasma tyrosine and urinary PHPAA was not significant. Of the four patients with tyrosyluria two were

Table 2. Initial and final values of plasma tyrosine (mmol/l) and urinary p-hydroxyphenyl lactic acid (PHPLA) and p-hydroxyphenyl acetic acid (PHPAA) ($\mu\text{g/ml}$) in marasmic and control patients aged 6 months–3 years given a high-protein diet alone or supplemented with ascorbic acid* for 7 d

Dietary regimen	No. of subjects	Plasma tyrosine		PHPAA		PHPLA	
		Mean	SD	Mean	SD	Mean	SD
High-protein diet	15						
Initial		0.11	0.04	24.3	21.6	5.4	4.5
Final		0.13	0.07	25.9	20.2	6.3	5.6
Statistical significance of difference between groups		NS		NS		NS	
High-protein diet + ascorbic acid	15						
Initial		0.09	0.06	17.6	14.9	5.3	7.3
Final		0.09	0.03	15.4	15.9	1.1	1.5
Statistical significance of difference between groups		NS		NS		$P < 0.05$	

* For details of dietary treatment, see p. 388.
NS, not significant ($P > 0.05$).

Table 3. Initial and final values of plasma tyrosine (mmol/l) and urinary p-hydroxyphenyl lactic acid (PHPLA) and p-hydroxyphenyl acetic acid (PHPAA) ($\mu\text{g/ml}$) in four marasmic patients aged 6 months–3 years with tyrosyluria, two of whom were given a high-protein diet alone or supplemented with ascorbic acid* for 7 d

Dietary treatment	Patient	Plasma tyrosine		PHPAA		PHPLA	
		Initial	Final	Initial	Final	Initial	Final
High-protein diet	1	0.08	0.13	10.0	13.3	16.6	17.0
	2	0.08	0.09	10.0	13.3	13.3	16.6
High-protein diet + ascorbic acid	1	0.09	0.09	50.0	50.0	25.0	Nil
	2	0.28	0.11	13.3	8.3	17.0	0.8

* For details of dietary treatment, see p. 388.

allocated to each group. In the two patients given the high-protein diet alone there was a small but insignificant rise in all values. In the two patients given supplementary ascorbic acid there was a very marked reduction in PHPLA and a reduction in plasma tyrosine and urinary PHPAA in one patient, with no change in either value in the other patient.

DISCUSSION

In a study on neonates Saini *et al.* (1975) reported that only 10% excreted large amounts of PHPLA and PHPAA (a urinary excretion of more than 10 $\mu\text{g/ml}$ was considered high excretion). These subjects were considered to be deriving their aromatic acids from PHPPA and so were conventional cases of tyrosyluria. A further 60% of the subjects were found to have an increased excretion of PHPAA which was thought to be derived from the pathway: tyrosine \rightarrow tyramine \rightarrow PHPAA. The latter subjects were considered to have high PHPAA excretion based on the findings of Saini *et al.* (1970) in 10% of normal adults. In the present study only 30% of normal children between 6 months and 3 years were found to have high PHPAA excretion, suggesting that high excretion of PHPAA is age-related. On the other hand 53.3% of marasmic children in the same age group excreted large

amounts of PHPAA. Thus high excretion of PHPAA is not only related to age but also to the nutrition of the child.

None of the controls in the present study was found to have a conventional type of tyrosyluria. Although plasma tyrosine and urinary PHPLA and PHPAA levels were significantly increased in marasmic patients compared to the controls, only four patients could be described as having tyrosyluria; all four patients had increased PHPLA and PHPAA excretion but only one patient had increased plasma tyrosine levels (0.28 mmol/l). This may be due to inadequate protein intake in the patients at admission.

In order to determine whether deranged tyrosine metabolism in marasmus is due to the deficiency of tyrosine metabolizing hepatic enzymes or of ascorbic acid needed to maintain normal activity of PHPPA-oxidase one group of patients was given a high-protein diet while the other was also given supplementary ascorbic acid. In the group given ascorbic acid there was a significant reduction in PHPLA excretion, the fall being very marked in two patients with tyrosyluria. The fall in PHPLA excretion suggested that tyrosyluria in these two patients was due to the ascorbic acid deficiency, which also caused the increased excretion of PHPLA generally observed in marasmic patients (though clinical scurvy was not evident). There was no fall in PHPAA excretion; this is probably due to the fact that PHPLA arises from PHPPA which accumulates in these patients due to reduced activity of PHPPA-oxidase, but PHPAA also arises from tyramine (tyrosine \rightarrow tyramine \rightarrow PHPAA), a pathway which is unaffected by ascorbic acid (Aggarwal *et al.* 1973).

In the group of patients given the high-protein diet alone there was no significant increase in either plasma tyrosine or urinary excretion of PHPLA and PHPAA. In the two patients with tyrosyluria there was a slight increase in these values, but this was not significant. It is possible that there is only a partial reduction in the activity of hepatic PHPPA-oxidase.

There are four components of tyrosyluria: raised plasma tyrosine and increased urinary excretion of PHPPA, PHPLA and PHPAA. In the present study PHPPA was not estimated because it is a labile compound which is easily destroyed by extraction and chromatographic separation under alkaline solutions. Increased excretion of PHPAA alone cannot be taken as an indicator of tyrosyluria because a certain population of subjects normally excrete large amounts of this phenolic acid; in our patients plasma tyrosine did not show a consistent rise. The increase is probably due to the fact that in marasmus PHPPA-oxidase is only partially affected and tyrosine transaminase which is not dependent on ascorbic acid for its activity, is normal. Only urinary PHPLA, which is derived from PHPPA (an important intermediary in tyrosine metabolism), has proved to be a good index of tyrosyluria.

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