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TYROSINEMIA AND TYROSYLURIA IN HEALTHY PREMATURES: TIME COURSES NOT VITAMIN C-DEPENDENT

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Summary

Tyrosyluria and for a part also tyrosinemia were studied in 60 healthy prematures of various birth weights and gestational ages. The first analyses were performed between the 6th and the 14th day after birth. A normal milk diet was given and the protein-intake was between 3 and 4 g/kg. After the first collection of urine half the patients received extra ascorbic acid, 100 mg/kg daily.

Urinary analyses of tyrosine and *p*-hydroxyphenyl metabolites were performed once a week, until the excretion of *p*-hydroxyphenylpyruvic plus *p*-hydroxyphenyllactic acids was lower than 5 mmoles per gram creatinine.

In 22 out of the 60 prematures (or 37%) a tyrosyluria of more than 5 mmoles/g creatinine and in 19 out of 44 (43%) patients analysed serum tyrosine was higher than 5 mg/100 ml at first analysis.

No inverse correlation between tyrosyluria and tyrosinemia on the one hand and birth weight and gestational age on the other hand existed. But in children with a delayed intra-uterine development the incidence of tyrosyluria was higher as prematurity was more pronounced.

Ascorbic acid had no effect on the rate of disappearance of tyrosyluria. It was concluded that the addition of extra vitamin C to the diet of prematures is not useful for the normalization of tyrosine metabolism.

Introduction

Many prematures and even a number of newborns excrete excessive amounts of tyrosine and its *p*-hydroxyphenyl metabolites at a protein-intake of

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more than 5 g/kg/day [1—5]. Also increased serum tyrosine concentrations, even higher than 16 mg/100 ml were observed [6,7].

According to the literature the administration of an excess of ascorbic acid prevents or diminishes this metabolic abnormality, at least in a part of the patients [3,8,9] but not in all [10—13]. Although tyrosinemia and tyrosyluria occur in vitamin-C deficiency, this is not the cause of the transient abnormality of tyrosine metabolism at an early age. In tyrosinemic children a normal leucocyte ascorbate concentration was found [14].

Also at a lower protein-intake hypertyrosinemia (serum tyrosine between 2 and 10 mg/100 ml) and tyrosyluria were observed [14]. Some investigators found little relationship between the incidence of transient hypertyrosinemia (excessive or moderate) and birth weight or gestational age [7,14]. But still other investigators did establish such a relationship [9—11,13,15].

The enzyme defect underlying neonatal tyrosinemia is complicated. It was suggested that except for a low *p*-hydroxyphenylpyruvic acid hydroxylase activity (EC 1.13.11.27) also substrate inhibition of this enzyme occurs [16—22]. Ascorbic acid is considered to be an important cofactor [3,8,9]. Whether tyrosine accumulation is accompanied by clinical symptoms is not yet clear, although lethargy has been observed in prematures with tyrosinemia [10,23—25]. Intellectual development of prematurely born children with hypertyrosinemia was normal and neurological disturbances in these children did not occur more frequently than in controls [23,26—28]. On the other hand there are indications that moderate mental retardation may result from prolonged neonatal hypertyrosinemia [29,30]. Permanent tyrosinemia and tyrosyluria in combination with mental retardation has been described [31—35].

The present work is a quantitative study of tyrosyluria and tyrosinemia in healthy prematures on a normal protein-intake of 3—4 g/kg/24 h. We wanted to investigate whether this phenomenon might be correlated with birth weight, gestational age or intra-uterine development. Is it possible to correct this biochemical abnormality with high supplements of ascorbic acid or to influence with ascorbic acid the time course of its disappearance.

Materials and Methods

Laboratory methods

For orientation of urinary amino acids small scale two-dimensional thin layer chromatography (on 5 cm × 5 cm chromatograms) was used [36]. Quantitative amino acid analysis was done by automated ion exchange column chromatography (Technicon TSM, apparatus; standard method for physiological fluids). Also a rapid TSM 1 procedure (66 minutes) for serum tyrosine and methionine was used. Resin bed 25.5 cm; column diameter 0.4 cm; lithium/citrate buffer, pH 3.25; Li 0.50 *N*; citrate 0.05 M; pump rate 0.45 ml/min. For orientation of urinary phenolic acids the method of Armstrong et al. [37] was used, but two-dimensional paper chromatography was replaced by thin-layer chromatography (D.C. Alufolien cellulose 0.1 mm, E. Merck A.G., Darmstadt No. 5552) 10 cm × 10 cm. The first solvent was isopropanol/ammonia 5% (8 : 2); the second one benzene/glacial acetic acid/water (125 : 72 : 3). Quantitative determinations of phenolic acids were performed by gas chro-

matography of their trimethylsilyl derivatives as described previously [38]. However, in this study the temperature programming was modified: 75° C for 10 minutes followed by an increase of 2° C per minute up to 220° C and finally 10 minutes at 220° C.

Patients and investigations

We studied 60 prematures of different birth weight, gestational age and intra-uterine development. See the Tables I and II. Urine (12 h) was collected between the 6th and the 14th day of life.

Collection of urine was started when physiological hyperbilirubinaemia and eventual respiratory distress had disappeared. Only patients in whom neonatal sepsis had been excluded were studied.

After collection of the first urine, half of the patients received extra ascorbic acid (100 mg/kg/24 h in 4 doses) during the remaining period of the investigations. No extra vitamin C was given to the others. Urinary tyrosine and *p*-hydroxyphenyl metabolites were determined once a week in both groups of children until the tyrosyluria (the sum of all *p*-hydroxyphenyl metabolites determined) was less than 5 mmol/g creatinine.

TABLE I

30 PREMATURES WHO RECEIVED EXTRA VITAMIN C AFTER THE FIRST URINE COLLECTION

Patient No.	Name	Sex	Birth weight (g)	Gestation (weeks)	Percentile Lubchenco
1	C.V.	M	950	32 + 5 days	<10
2	T.O.	M	1170	32	<10
3	L.N.	F	1280	33	<10
4	J.E.	M	1370	29 + 1 day	50—75
5	R.v.d.B.	M	1440	35 + 2 days	<10
6	M.v.d.N.	M	1520	33	10—25
7	M.K.	F	1660	34 + 5 days	<10
8	E.L.	F	1760	34 + 2 days	10—25
9	S.D.	F	1830	37	<10
10	A.G.	M	1840	34 + 4 days	10—25
11	J.P.	M	1880	32 + 2 days	50—75
12	T.E.	F	1880	37 + 6 days	<10
13	A.B.	F	1920	37	<10
14	A.H.	F	2000	35	10—25
15	I.K.	M	2020	35	10—25
16	S.W.	F	2100	33 + 5 days	25—50
17	L.M.	M	2100	36	10—25
18	E.v.d.B.	M	2120	34 + 1 day	25—50
19	S.H.	F	2130	35	10—25
20	C.K.	F	2200	34 + 5 days	25—50
21	L.v.R	F	2240	35 + 1 day	25—50
22	D.v.B	F	2250	35 + 6 days	10—25
23	S.R.	F	2260	34 + 3 days	25—50
24	L.v.B.	F	2280	35 + 6 days	10—25
25	M.S.	M	2310	34 + 3 days	50—75
26	H.D.	M	2360	35	25—50
27	M.B.	M	2390	36	10—25
28	E.E.	M	2450	35	25—50
29	K.D.	F	2500	37	10—25
30	L.M.	M	2520	36	25—50

TABLE II

30 PREMATURES WHO RECEIVED NO EXTRA VITAMIN C

Patient No.	Name	Sex	Birth weight (g)	Gestation (weeks)	Percentile Lubchenco
1	F.T.	M	1320	33 + 5 days	<10
2	F.W.	M	1480	31	25—50
3	J.M.	M	1480	32	10—25
4	F.T.	F	1600	33 + 5 days	10—25
5	P.v.R.	M	1600	35 + 3 days	<10
6	P.A.	F	1640	31 + 3 days	50—75
7	S.B.	F	1720	34	10—25
8	A.v.B	F	1720	40	<10
9	R.S.	M	1750	36	<10
10	P.v.R.	F	1760	36	<10
11	R.J.	M	2020	33—34	25—50
12	J.K.	F	2030	31 + 3 days	75—90
13	P.L.	M	2030	35 + 1 day	10—25
14	A.H.	F	2090	34	25—50
15	R.J.	F	2120	34	25—50
16	J.E.	F	2180	34 + 2 days	25—50
17	F.M.	F	2180	36 + 5 days	<10
18	V.B.	M	2200	32	75—90
19	W.K.	M	2270	34 + 5 days	25—50
20	R.d.R.	M	2340	36 + 2 days	10—25
21	M.S.	M	2380	33 + 6 days	50—75
22	C.K.	M	2390	35	25—50
23	T.v.R.	M	2430	35 + 3 days	10—25
24	E.D.	M	2450	35	25—50
25	H.v.d.B.	M	2500	34 + 4 days	50—75
26	V.C.	M	2500	36 + 2 days	25—50
27	F.M.	F	2520	36 + 5 days	10—25
28	L.R.	F	2560	35 + 3 days	25—50
29	P.D.	F	2600	35	50—75
30	E.A.	M	2620	35 + 4 days	25—50

In 44 out of the 60 patients serum tyrosine, methionine and other amino acids were determined after the first collection of urine, just before feeding. In a number of cases amino acids were determined retrospectively for reasons of completeness.

All the children were fed on a relatively low protein milk diet, containing 3.5 (±0.5) g protein per kg per 24 h, corresponding with 158 mg tyrosine and 161 mg phenylalanine/kg/24 h. Basal dietary vitamin C, as calculated, was 10 mg/kg/24 h.

Results

1. Frequency of tyrosyluria in both groups of patients

Urinary data in 30 patients with extra vitamin C are given in Table III; those in the 30 control patients can be seen in Table IV.

The Figs 1 and 2 show the course of the tyrosyluria (*p*-hydroxyphenyllactic acid + *p*-hydroxyphenylpyruvic acid (*p*-OHPLA + *p*-OHPPA), expressed as mmol/g creatinine). Only children with initial values higher than 5 mmoles/g creatinine were considered.

TABLE III

SERUM TYROSINE, SERUM METHIONINE, URINARY TYROSINE, *p*-HYDROXYPHENYLLACTIC ACID (*p*-OHPLA), *p*-HYDROXYPHENYLPYRUVIC ACID (*p*-OHPPA), *p*-HYDROXYPHENYLACETIC ACID (*p*-OHPAA), TOTAL *p*-HYDROXYBENZOIC ACID (*p*-OHBA), TOTAL *o*-HYDROXYBENZOIC ACID (*o*-OHBA), TOTAL BENZOIC ACID (BA) AND TOTAL PHENYLACETIC ACID (PA) IN 30 PREMATURES WITH EXTRA VITAMIN C

Patient No.	Name and birth date	Date of sampling	Weight (g)	Serum (mg/100ml)			Urine (mmol/g creatinine)							
				Tyrosine	Methionine	Creatinine (mg/l)	Tyrosine	*p*-OH PLA	*p*-OH PPA	*p*-OH PAA	*p*-OH BA	*o*-OH BA	BA	PA
1	C.V.	10- 1-72	970	27,3	0,7	83	19,9	28.3	3,5	7,4	2,1	3,8	1,6	0,5
	28-12-71	14- 1-72	1080			65		48.6	3,2	6,8	1,5	2,8	1,4	0,4
		21- 1-72	1280			70		47,0	6,0	7,7	2,3	2,1	tr	0,6
		28- 1-72	1525			91		36,4	4,5	8,0	1,8	2,2	1,0	0,5
		4- 2-72	1680	13,2	0,7	69		25,3	1,9	4,8	2,3	2,9	2,1	0,8
		11- 2-72	1920			60		2,2		2,3	0,9	tr	0,8	1,0
		21- 2-72	2140			80		0,5		1,1	0,6	0,1	0,5	0,5
		28- 2-72	2340	3,3	0,8	60		0,5		0,8	0,6	0,8	0,6	1,1
2	T.O.	17- 4-72	1250	32,6	1,2	65	6,2	50,0	6,1	5,3	2,0	4,0	tr	tr
	7- 4-72	24- 4-72	1560			75		46,7	2,8	7,4	1,3	1,7	1,0	0,4
		28- 4-72	1660			75		47,7	2,8	8,2	1,5	1,5	1,2	0,7
		2- 5-72	1830			85		46,4	4,6	8,2	1,2	0,8	1,0	0,4
		5- 5-72	1920			85		40,7	6,3	5,3	1,1	2,2	0,9	0,3
		12- 5-72	2140			90		48,1	7,6	2,8	0,8	2,1	0,2	0,2
		19- 5-72	2380			100		37,6	8,7	4,5	0,9	2,3	0,4	0,8
		26- 5-72	2590	17,0	0,6	110	2,0	29,6	7,0	4,1	0,7	2,2	0,2	0,6
		29- 5-72	2710			215		24,5	5,9	1,8	0,8	1,1	0,1	0,3
		5- 6-72	2950			125		14,4	4,8	2,5	0,7	1,5	0,1	0,3
		12- 6-72	3170			120		1,6	1,0	1,0	0,5	2,0	0,1	1,1
3	L.N.	10- 1-72	1470	4,1	0,6	98	0,4	0,5		0,7	0,5	1,4	0,4	0,2
	23-12-71	14- 1-72	1570			85		5,9	0,5	1,6	0,6	1,6	0,5	0,3
		21- 1-72	1800			86		1,1		1,0	1,0	1,5	1,5	0,8
4	J.E.	29-10-71	1260			94	0,2	0,5	0,1	0,3	0,7	1,6	0,4	0,6
	17-10-71													
5	R.v.d.B.	4- 9-72	1520	18,0	0,7	95	2,2	31,3	5,1	2,7	0,5	0,7	0,5	0,2
	27- 8-72	10- 9-72	1640			135		37,4	5,6	6,8	0,7	2,1	0,7	0,4
		17- 9-72	1790			115		11,2	0,1	3,7	0,9	1,8	0,8	0,8
		24- 9-72	2060			55		7,6	1,2	4,0	0,8	1,7	1,1	2,2
		1-10-72	2360			45		19,9	4,2	3,0	1,0	2,2	0,8	0,3
		9-10-72	2620			50		2,1	tr	1,2	0,6	1,9	0,6	0,2

TABLE III (continued)

TABLE III (continued)

Patient No.	Name and birth date	Date of sampling	Weight (g)	Serum (mg/100ml)			Urine (mmol/g creatinine)						
				Tyrosine	Methionine	Creatinine (mg/l)	Tyrosine	*p*-OH PLA	*p*-OH PPA	*p*-OH PAA	*p*-OH BA	*o*-OH BA	BA PA
6	M.v.d.N.	25- 9-72	1630	28,0	1,3	55	2,7	36,3	2,0	9,1	1,2	4,1	1,0 0,7
	11- 9-72	2-10-72	1910			55		38,5	1,0	7,9	0,8	1,8	1,4 1,6
		9-10-72	2170	11,9	0,7	60	1,8	19,0	0,6	7,2	0 9	1,5	0,8 1,5
		16-10-72	2330			95		3,2	0,1	2,2	0,9	0,6	1,0 4,5
7	M.K. 2-11-72	10-11-72	1620			75	tr	1,5	0,3	0,2	0,3		0,4 0,2
8	E.L. 26- 1-72	2- 2-72	1800			67		0,7	0,3	0,8	0,5		0,5 0,6
9	S.D.	11- 8-72	1900	9,5	0,4	100	1,0	10,3	2,2	0,7	0,5	1,6	1,0 0,1
	5- 8-72	21- 8-72	2100			110		1,8	0,1	0,8	0,5	2,1	0,1 5,3
10	A.G.	12-10-72	1920	15,9	0,9	100	0,7	11,5	2,3	3,4	0,3		0,4 0,3
	6-10-72	24-10-72	2110	12,6	1,0	95		18,7	2,7	1,8	0,7		0,5 0,9
		31-10-72	2300			85		1,5		0,9	0,4		0,5 0,9
		7-11-72	2600	4,5	0,8	110		0,4	0,4	0,5	0,4		0,5 0,8
11	J.P. 18-10-71	29-10-71	1750			126		3,9	0,3	1,2	0,8	1,9	0,5 2,8
12	T.E. 8- 9-72	24- 9-72	2160	3,8	1,0	65	0,5	0,4		0,5	1,2	0,7	2,8 0,2
13	A.B. 29-11-71	7-12-71	2020			115		1,2	tr	0,5	0,3		0,3 0,4
14	A.H.	19- 9-72	2170	4,7	0,7	160		0,8		0,7	0,2		0,4 0,5
	9- 9-72	26- 9-72	2270			115		0,4	0,1	0,5	0,5		0,6 0,3
		3-10-72	2620	2,5	0,9	85		0,2		0,5	0,2		0,7 0,7
15	J.K. 18-10-72	25-10-72	2120	2,8	1,0	90	0,3	1,2	0,3	0,4	0,2		0,4 0,2
16	S.W.	3-11-72	2070	3,6	0,8	90	0,2	0,3	tr	0,2	0,3		0,4 3,9
	27-10-72	12-11-72	2250	3,4	0,9	105	0,6	0,1	tr	0,6	0,2		0,4 1,2
17	L.M.	11-11-72	1990			110		0,6	0,4	0,2	0,3		0,3 0,2
	7-11-72	17-11-72	2120	2,5	0,9	135	0,2	0,3		0,3	0,3		0,3 0,4

18	E.v.d.B.	12-10-72	2100	8,2	1,0	80	0,8	10,8	1,5	2,0	0,7		0,6	0,9
	3-10-72	24-10-72	2410			95		0,5	0,4	0,5	0,3		0,5	0,7
		31-10-72	2690			70		0,2	tr	0,6	0,3		0,4	0,8
		7-11-72	3000	3,3	0,8	110	0,3	0,1	0,2	0,5	0,3		0,4	0,7
19	S.H.	8- 8-72	2130	4,4	0,6	110	0,2	0,5	tr	0,1	0,1		0,6	3,0
	1- 8-72													
20	C.K.	10-11-72	2070			110	0,3	0,7	0,3	0,5	0,2		0,4	0,5
	2-11-72													
21	L.v.R.	2- 2-72	2160			236		1,2	0,3	0,6	0,2		0,2	0,5
	24- 1-72													
22	D.v.B	28- 9-72	2320	3,2	1,0	95	0,4	1,0	0,4	0,3	0,3		0,4	0,1
	15- 9-72													
23	S.R.	1- 8-72	2300	25,6	1,0	110	3,0	46,3	9,3	1,4	0,7		0,6	0,3
	22- 7-72	8- 8-72	2440	2,9	0,8	145		0,6	0,3	0,3	0,3		0,4	0,3
24	L.v.B.	28- 9-72	2330	3,3	1,0	180		0,7	0,5	0,3	0,3		0,4	0,2
	15- 9-72													
25	M.S.	14- 1-72	2400			302		0,7		0,2	0,4		tr	0,1
	4- 1-72													
26	H.D.	20-11-72	2290	5,8	1,1	95	1,5	19,4	2,7	4,0	0,6	1,7	0,4	4,3
	11-11-72	27-11-72	2540			95		0,3		0,6	0,4	1,3	0,3	0,3
		4-12-72	2960	2,2	1,0	105	0,5	0,2		0,7	0,3	1,2	0,3	0,3
27	M.B.	14- 1-72	2410			71		2,0	0,8	1,3	0,9		tr	0,3
	7- 1-72													
28	E.E.	14- 1-72	2310			137		49,8	8,3	1,5	1,2		tr	tr
	4- 1-72	21- 1-72	2340	1,5	0,4	84		0,3		0,5				
29	K.D.	11- 8-72	2550	19,0	0,4	100	1,8	17,2	4,5	1,6	1,3	1,3	0,6	0,1
	5- 8-72	21- 8-72	2800			100		0,7	0,1	0,7	0,4	1,0	0,4	0,7
30	L.M.	11-11-72	2410	3,6	0,4	150	1,4	24,4	4,7	1,1	0,4		0,3	0,3
	7-11-72	17-11-72	2540	4,9	0,7	150	0,7	7,1	0,5	1,4	0,4		0,3	0,9
		24-11-72	2660	2,9	0,9	135	0,2	0,3	0,1	0,4	0,2		0,3	0,4

TABLE IV

SERUM TYROSINE, SERUM METHIONINE, URINARY TYROSINE, *p*-HYDROXYPHENYLLACTIC ACID (*p*-OHPLA), *p*-HYDROXYPHENYLPYRUVIC ACID (*p*-OHPPA), *p*-HYDROXYPHENYLACETIC ACID (*p*-OHPAA), TOTAL *p*-HYDROXYBENZOIC ACID (*p*-OHBA), TOTAL *o*-HYDROXYBENZOIC ACID (*o*-OHBA), TOTAL BENZOIC ACID (BA) AND TOTAL PHENYLACETIC ACID (PA) IN 30 PREMATURES WITHOUT EXTRA VITAMIN C

Patient No.	Name and birth date	Date of sampling	Weight (g)	Serum (mg/100 ml)			Urine (mmol/g creatinine)							
				Tyrosine	Methionine	Creatinine (mg/l)	Tyrosine	*p*-OH PLA	*p*-OH PPA	*p*-OH PAA	*p*-OH BA	*o*-OH BA	BA	PA
1	F.T.	7- 3-72	1410	25,5	0,8	100	1,1	49,4	4,2	3,9	1,3		1,3	0,9
	25- 2-72	15- 3-72	1560			110		41,9	4,6	2,9	2,1		tr	1,6
		21- 3-72	1730			105	1,3	50,7	4,4	4,0	1,7		1,7	0,6
		29- 3-72	2000			130	1,2	34,5	3,8	1,9	1,1		1,0	0,9
		5- 4-72	2180			185		7,7	0,6	1,7	0,9		1,0	1,7
		13- 4-72	2510			115		5,8	0,4	2,0	0,8		1,0	0,8
		19- 4-72	2770	6,6	0,8	150	1,0	5,4		1,7	0,7		0,4	1,3
		27- 4-72	3200			100		1,7	0,3	1,2	0,4		0,4	1,0
2	F.W.	19- 7-72	1720	8,1	0,6	100	0,8	9,2	0,4	0,9	0,5		0,7	0,4
	7- 7-72	4- 8-72	1930	4,6	1,0	85	0,3	2,1		0,9	0,2		0,5	0,5
3	J.M.	27-11-72	1410	3,5	0,7	95	0,4	4,0	0,5	0,3	0,5		0,3	2,5
	16-11-72	4-12-72	1630	3,0	0,6	80	0,4	0,6		0,9	0,9	1,8	0,5	0,4
4	F.T.	7- 3-72	1600	29,5	1,0	80	3,1	76,6	4,2	6,6	1,8		2,0	2,0
	25- 2-72	15- 3-72	1820			120		53,8	4,6	2,6	1,9		tr	4,9
		21- 3-72	1950			85	2,7	45,6	4,7	3,0	1,7		1,9	1,2
		29- 3-72	2170			65	3,6	38,7	3,8	2,8	2,0		2,0	1,1
		5- 4-72	2350			80		3,0		1,3	1,4		1,8	1,5
		13- 4-72	2640			115		1,6	tr	1,0	0,8		1,2	0,6
		19- 4-72	2870	4,0	1,0	175	0,5	1,0	0,1	0,7	0,4		0,5	0,5
5	P.v.R.	5- 7-72	1610	1,2	0,3	95	1,7	25,8	2,9	1,3	0,5		0,4	0,1
	27- 6-72	12- 7-72	1800	38,6	1,5	85	4,5	35,4	7,3	1,2	0,7		0,6	0,3
		20- 7-72	2040	22,8	0,8	95	1,8	33,6	6,8	1,7	0,7		0,5	0,1
		26- 7-72	2170			90		17,0	4,0	1,3	0,6		0,5	0,4
		1- 8-72	2410	9,3	0,9	65	1,3	11,6	1,0	0,9	0,8		0,6	0,4
		8- 8-72	2640			250		0,6	0,2	0,1	0,3		0,3	0,5
6	P.A.	1- 6-72	1630			85		1,0		0,4	0,2		0,4	0,8
	19- 5-72													

7	S.B.	26- 5-72	1640	9,7	0,9	90	0,6	2,3	0,3	0,3	0,4	tr	0,4	2,9
	20- 5-72													
8	A.v.B.	29- 6-72	1780			130		29,7	7,0	1,3	0,5		0,3	0,2
	18- 6-72	7- 7-72	1900	24,6	1,2	110	2,3	34,9	3,0	3,3	0,8		0,5	0,3
		14- 7-72	2070	9,7	1,0	110		7,5	1,2	0,8	0,9		0,6	0,3
		21- 7-72	2300	1,0	0,2	90		0,4	0,1	0,5	0,3		0,8	0,4
9	R.S.	28- 4-72	1730			200		0,2	tr	0,2	0,3	tr	0,3	0,1
	22- 4-72													
10	P.v.R.	5- 7-72	1900			75		43,8	9,6	1,6	0,8		0,4	0,4
	25- 6-72	12- 7-72	2100	12,3	1,0	100	1,3	24,6	2,2	1,0	0,9		0,7	0,3
		20- 7-72	2310	2,4	0,8	55		0,4		0,4	0,2		0,9	0,4
11	R.J.	12- 5-72	2030	31,8	0,4	85	1,6	42,4	4,2	2,3	0,8		0,1	0,1
	5- 5-72	19- 5-72	2270			90		33,4	2,2	2,6	1,0	2,7	0,2	0,8
		26- 5-72	2480			125		9,4	1,1	1,4	1,0	2,0	0,1	1,2
		29- 5-72	2580			95		7,2	0,3	1,6	0,8	2,4	0,1	0,3
		5- 6-72	2780			95		6,5	0,2	1,9	0,5	tr	0,2	0,1
		12- 6-72	3030			135		1,0	0,1	0,4	0,6	0,5	0,4	0,4
12	J.K.	24- 2-72	1940	1,9	0,5	80	0,3	0,6		0,3	0,4	0,9	0,5	2,4
	14- 2-72													
13	P.L.	29- 7-72	2350	2,4	0,9	115		0,1		0,5	0,2		0,6	0,4
	11- 7-72													
14	A.H.	26- 5-72	2000	1,4	0,5	115		1,8	0,1	0,1	0,4		0,2	0,8
	19- 5-72													
15	R.J.	15- 5-72	2300	2,5	0,6	175		0,2	tr	0,3	0,3	tr	0,2	0,4
	2- 5-72													
16	J.E.	29- 3-72	2250			80		0,5		3,0	0,3		0,6	5,1
	22- 3-72													
17	F.M.	19- 9-72	2320	3,8	0,7	130		0,9	tr	0,3	0,2		0,3	0,3
	9- 9-72													
18	V.B.	4- 6-72	2070	2,8	0,8	110		1,8	0,3	0,4	0,4	0,6	0,2	0,2
	25- 5-72													
19	W.K.	14- 5-72	2420	2,9	0,7	105		0,8		0,3	0,2		0,2	0,4
	28- 4-72													
20	R.d.R.	13- 4-72	2440			70		tr		0,4	0,4		0,7	0,3
	1- 4-72													

TABLE IV (continued)

TABLE IV (continued)

Patient No.	Name and birth date	Date of sampling	Weight (g)	Serum (mg/100 ml)			Urine (mmol/g creatinine)							
				Tyrosine	Methionine	Creatinine (mg/l)	Tyrosine	*p*-OH PLA	*p*-OH PPA	*p*-OH PAA	*p*-OH BA	*o*-OH BA	BA	PA
21	M.S.	29- 6-72	2300			70	2,0	39,2	7,9	2,0	1,2		2,9	0,2
	21- 6-72	7- 7-72	2530	2,9	0,8	95	0,4	1,9	tr	0,5	0,4		0,3	0,7
22	C.K.	29- 6-72	2410	1,6	0,7	80		1,0	0,2	0,4	0,4	0,2	0,5	0,5
	19- 6-72													
23	T.v.R.	5- 7-72	2470	7,0	0,8	100		0,9	tr	0,6	0,3		0,4	0,4
	27- 6-72													
24	E.D.	1- 6-72	2420	11,3	0,5	155	0,8	13,6	0,9	0,8	0,5		0,1	0,1
	23- 5-72	9- 6-72	2640	3,7	1,1	240		0,2	0,1	0,2	0,2		0,2	0,3
25	H.v.d.B.	7- 3-72	2600	3,0	0,7*	90		0,8		0,5	0,4	tr	0,6	0,3
	24- 2-72													
26	V.C.	29- 3-72	2390			160		0,3		0,4	0,3	0,2	0,4	0,2
	23- 3-72													
27	F.M.	19- 9-72	2650	3,6	0,9	140		1,0	tr	0,2	0,2		0,3	0,2
	9- 9-72													
28	L.R.	1- 3-72	2480	2,6	0,5	200		0,7	0,2	0,2	0,4	tr	0,3	0,2
	22- 2-72													
29	P.D.	23- 8-72	2420	2,0	0,5	235		0,2	tr	0,7	0,2		0,5	0,9
	15- 8--72													
30	E.A.	15- 5-72	2500	6,1	0,7	180	0,3	6,7	0,5	0,4	0,4		0,1	0,6
	6- 5-72	24- 5-72	2730			145		0,8	tr	0,3	0,2		0,2	0,6

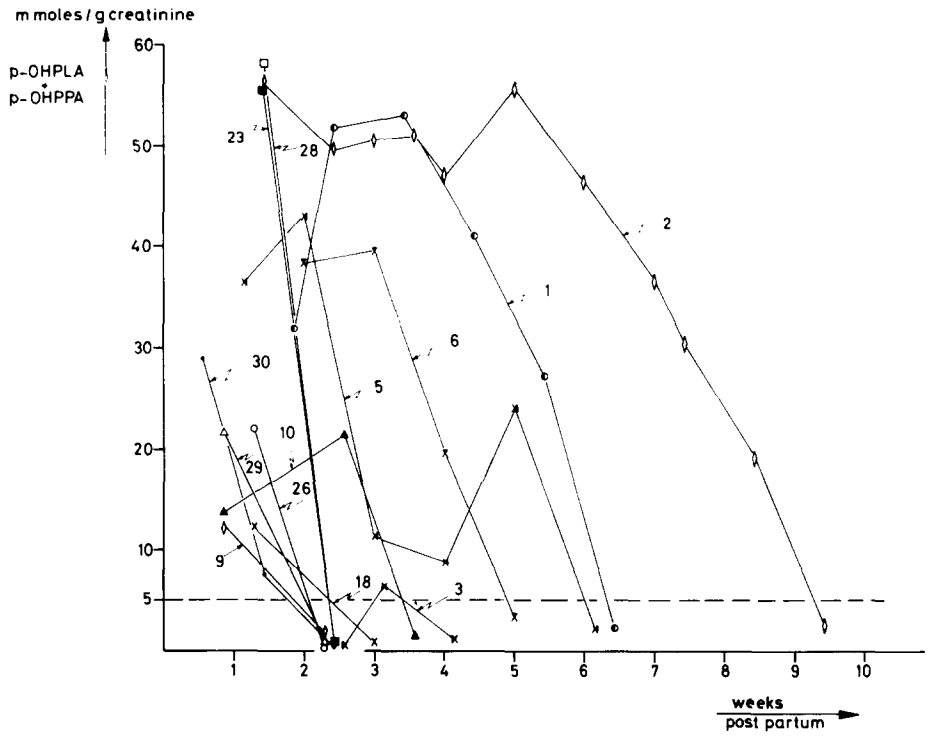

Fig. 1. Course of tyrosyluria (*p*-hydroxyphenyllactic acid (*p*-OHPLA) + *p*-hydroxyphenylpyruvic acid (*p*-OHPPA)) in 13 patients with extra vitamin C.

In 13 out of 30 children receiving extra vitamin C at least one excretory value exceeding 5 mmoles/g creatinine was found. In the controls this number was 10.

2. Quantitative and qualitative aspects

By definition urinary *p*-OHPLA + *p*-OHPPA is the parameter used for tyrosyluria. *p*-OHPLA contributes for some 80% to this quantity. See the Tables III and IV.

In our children the excretory level of *p*-OHPAA is (with some exceptions) equal to or lower than *p*-OHPPA. Although *p*-OHPAA can arise from oxydative decarboxylation of *p*-OHPPA, it was not used as a measure for tyrosyluria for reasons given below.

In most children with tyrosyluria urinary tyrosine is lower than or about equal to *p*-OHPPA. However, the initial urinary tyrosine concentration in patient C.V. (Table III) of 19.9 mmoles/g creatinine was exceptional. As the serum tyrosine of 27.3 mg/100 ml was not extremely high, we concluded that renal absorption was decreased. The child appeared to have a generalized renal amino aciduria. Serum methionine was slightly elevated; phenylalanine was normal. The protein-intake was 4.0 g/kg/24 h.

The interpretation of *p*-OHPAA is less easy, as this compound can both be of endogenous and of exogenous origin. Exogenous formation by bacterial

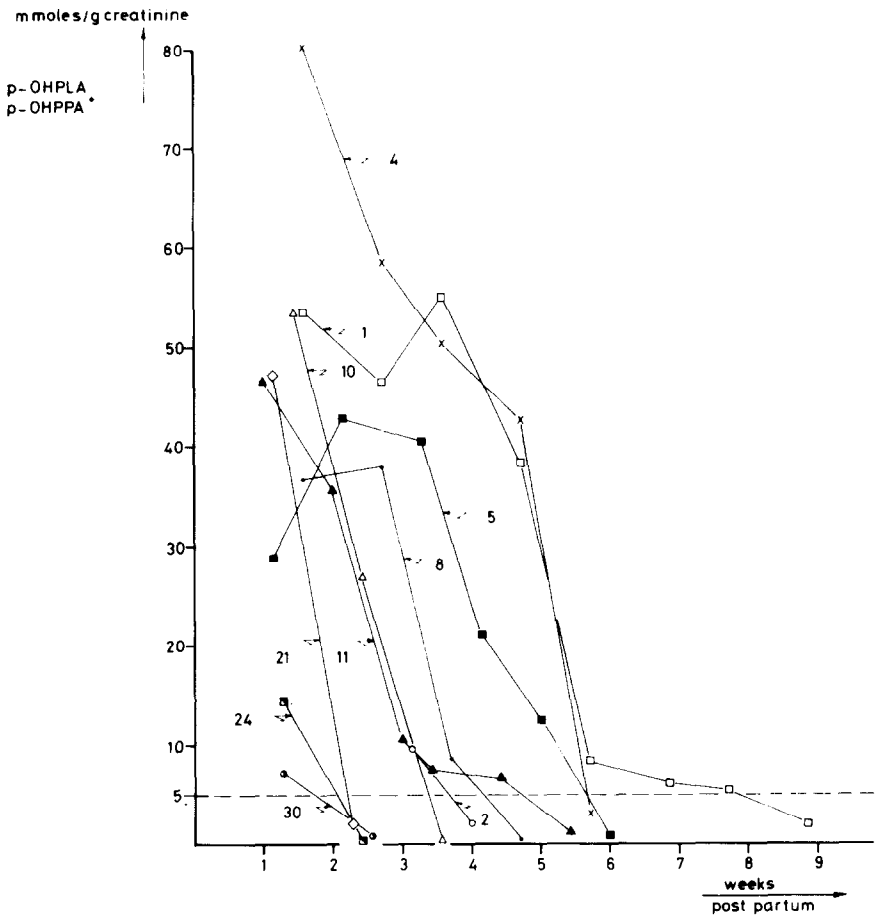

Fig. 2. Course of tyrosyluria (*p*-hydroxyphenyllactic acid (*p*-OHPLA) + *p*-hydroxyphenylpyruvic acid (*p*-OHPPA)) in 10 patients without extra vitamin C.

metabolism of non-absorbed tyrosine in the intestinal lumen is most likely (see van der Heiden et al. [39]).

In children of ½—13 years an upper normal limit of 0.67 mmole/g creatinine was found. Initially our prematures had much higher values, but this may partly be a consequence of a low creatinine in prematures. For another part (endogenous) *p*-OHPAA is higher when tyrosyluria is more pronounced at a younger age.

Para-hydroxybenzoic acid (*p*-OHBA) is also considered to be an exogenous metabolite of tyrosine, formed by the intestinal bacterial flora. In 18 children of ½—13 years the upper normal limit was 0.38 mmole/g creatinine [40]. In most prematures slightly elevated values were found up to 2.3 mmoles/g creatinine. A low creatinine production may rather be the cause than increased bacterial tyrosine metabolism in the intestine. On the other hand intestinal fluids will contain more *p*-hydroxyphenyl compounds in periods with tyrosine overflow. *p*-Hydroxybenzoic acid was highest when tyrosyluria was most pronounced.

Urinary benzoic acid is also supposed to be a bacterial metabolite of non-absorbed phenylalanine (or tyrosine). A part of the phenylacetic acid is also produced from phenylalanine by the intestinal bacteria.

Upper normal limits in children of ½—13 years are 1.53, respectively, 2.09 mmoles/g creatinine [40]. Slight elevation of urinary values in prematures (Tables III and IV) is presumably for the greater part due to a low creatinine production. In many prematures a strikingly elevated excretion of salicyluric acid was seen, which disappeared spontaneously. A highest value of 4.1 mmoles/g creatinine was found. In children with extra vitamin C the incidence of this phenomenon was more pronounced than in the controls. Up till now no explanation is available for the transient appearance of this compound.

Other urinary phenolic acids in prematures, established by 2-dimensional thin-layer chromatography.

Normal concentrations of 3-methoxy-4-hydroxy-phenylmandelic and 3-methoxy-4-hydroxy-phenylacetic acids (endogenous compounds) occurred in practically all urine samples. Ferulic and 4-methoxy-3-hydroxy-phenyl-hydracrylic acids (exogenous compounds) were practically always absent. In most samples 5-OHIAA was present. Only in 1 sample *o*-hydroxyphenylacetic acid was significantly increased. Meta-hydroxyphenyl compounds such as *m*-hydroxyphenylacetic acid, β-*m*-hydroxyphenylhydracrylic acid and *m*-hydroxy-hippuric acid seldom occur during the first weeks of life. These compounds are probably of exogenous origin and appear when a diet other than milk food is given.

Alpha-resorcylic acid, possibly also of exogenous origin was absent in all the children.

3. Correlations

From the data of Table V no inverse correlation between birth weight and tyrosyluria appeared. Nor did it appear between weeks of gestation and tyrosyluria (Table VI). But in children with a delayed intra-uterine development the incidence of tyrosyluria was higher as prematurity was more pronounced (Table VII). Serum tyrosine behaved in the same way with regard to the three parameters (see the Tables VIII, IX and X).

Furthermore, we observed that there was little correlation between serum tyrosine and the protein-intake (see Fig. 3a). Extra vitamin C seemed to have little effect on serum tyrosine (Fig. 3b). Great differences of serum tyrosine at

TABLE V

RELATIONSHIP BETWEEN BIRTH WEIGHT AND TYROSYLURIA (URINARY *p*-HYDROXYPHENYLLACTIC + *p*-HYDROXYPHENYLPYRUVIC ACIDS) AT THE FIRST DAY OF ANALYSIS IN 60 PREMATURES

Birth weight (g)	*p*-OHPLA + *p*-OHPPA (mmol/g creatinine)						
	0—5	6—15	16—25	26—35	36—45	46—55	⩾56
<1500	3	1		1	1	1	1
1500—2000	8	2		1	2	1	1
2000—2500	22	2	1			3	1
⩾2500	3	1	1	1			

TABLE VI

RELATIONSHIP BETWEEN THE WEEKS OF GESTATION AND TYROSYLURIA (URINARY *p*-HYDROXYPHENYLLACTIC + *p*-HYDROXYPHENYLPYRUVIC ACIDS) AT THE FIRST DAY OF ANALYSIS IN 60 PREMATURES

Gestation (weeks)	*p*-OHPLA + *p*-OHPPA (mmol/g creatinine)						
	0—5	6—15	16—25	26—35	36—45	46—55	⩾ 56
<32	5	1					1
<32—<34	3			1	1	3	1
⩽34—<36	21	4	1	1	1	1	1
⩾36	9	1	1	1	1	1	

TABLE VII

RELATIONSHIP BETWEEN THE INTRA-UTERINE DEVELOPMENT AND TYROSYLURIA (URINARY *p*-HYDROXYPHENYLLACTIC + *p*-HYDROXYPHENYLPYRUVIC ACIDS) AT THE FIRST DAY OF ANALYSIS IN 60 PREMATURES

Percentile (Lubchenco)	*p*-OHPLA + p-OHPPA (mmol/g creatinine)						
	0—5	6—15	16—25	26—35	36—45	46—55	⩾ 56
<10	6	1		2	2	2	1
10—25	14	1	1		1		1
25—50	10	4	1	1		2	1
50—75	6					1	
75—90	2						

TABLE VIII

RELATIONSHIP BETWEEN BIRTH WEIGHT AND SERUM TYROSINE AT THE FIRST DAY OF ANALYSIS IN 44 PREMATURES

Birth weight (g)	Serum tyrosine (mg/100 ml)						
	0—5	6—10	11—15	16—20	21—25	26—30	31—35
<1500	2	1		1	1	1	1
1500—2000	2	2	2		1	2	
2000—2500	16	2	1		1		1
⩾2500	5	1		1			

TABLE IX

RELATIONSHIP BETWEEN WEEKS OF GESTATION AND SERUM TYROSINE AT THE FIRST DAY OF ANALYSIS IN 44 PREMATURES

Gestation (weeks)	Serum tyrosine (mg/100 ml)						
	0—5	6—10	11—15	16—20	21—25	26—30	31—35
⩾32	3	1					1
<32—<34	3				1	3	1
⩽34—<36	14	4	2	1	1		
⩾36	5	1	1	1	1		

TABLE X

RELATIONSHIP BETWEEN INTRA-UTERINE DEVELOPMENT AND SERUM TYROSINE AT THE FIRST DAY OF ANALYSIS IN 44 PREMATURES

Percentile (Lubchenco)	Serum tyrosine (mg/100 ml)						
	0—5	6—10	11—15	16—20	21—25	26—30	31—35
<10	4	1	1	1	2	1	1
10—25	7	2	1	1		2	
25—50	10	3	1		1		1
50—75	3						
75—90	1						

a rather constant protein-intake point to individual differences of tyrosine metabolism. In 19 out of 44 first determinations serum tyrosine was higher than 5 mg/100 ml.

When investigating the relationship between urinary *p*-hydroxyphenyl compounds and serum tyrosine, we only considered the data of the first simultaneous analyses of serum and urine (see Tables III and IV).

Exceptional are the patients 26 and 30 (Table III) and patient 5 (Table IV), having only slightly elevated tyrosine levels and still relatively high excretions. This may indicate a strong catabolism of tyrosine via transamination. The

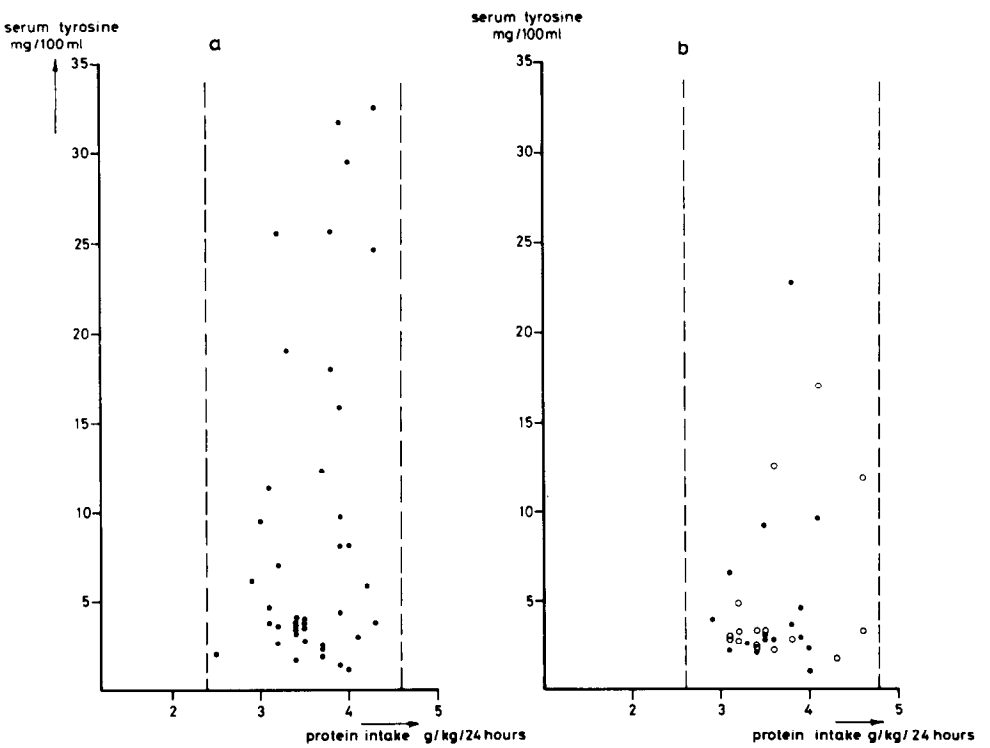

Fig. 3. a. Relationship between serum tyrosine and the protein-intake at the first day of analysis (before vitamin C administration). b. Relationship between serum tyrosine and the protein intake on the following days. ○, patients with extra vitamin C; ●, patients without extra vitamin C.

opposite is true for patient 7 (Table IV) with a rather high serum tyrosine level and a low urinary excretion of *p*-hydroxyphenyl compounds. Patient 1 (Table III), who excreted an exceptionally large amount of tyrosine, has already been mentioned. It is easy to see that tyrosyluria is predominantly determined by urinary *p*-hydroxyphenyllactic acid. This is the compound to be considered when urine is screened for tyrosyluria by 2-dimensional thin-layer chromatography of phenolic acids.

From the data of the Tables III and IV it can be concluded that no relationship exists between the increase of serum methionine and of serum tyrosine in healthy prematures. Serum methionine was elevated in many patients but at a lower level than was serum tyrosine. In general the decrease of serum methionine followed a time course different from that of serum tyrosine.

From Fig. 1 it can be seen that the 4 (out of 13) most abnormal curves (decrease below the 5 mmole limit after minimally 4 weeks) refer to children with a low percentile and a low birth weight of 1520 g or less. In Fig. 2 we see the same phenomenon. Three patients out of 10 with the most abnormal curves also had low percentile values and low birth weights of 1600 g or less. This points to a weak correlation with prematurity.

Vitamin C had little effect. From the Figs 1 and 2 it can be concluded that vitamin C has no favourable effect on the disappearance of the tyrosyluria (*p*-OHPLA + *p*-OHPPA as mmoles/g creatinine) in patients with excretions higher than 5 mmoles/g creatinine. Neither could an effect on serum tyrosine be discovered (see Fig. 3b).

Discussion

We investigated tyrosyluria in 60 healthy prematures on a moderate protein-intake of 3—4 g/kg/day. At first analysis, between the 6th and the 14th day of life, in 22 of them or in 37% tyrosyluria (urine *p*-OHPLA + *p*-OHPPA) was more than 5 mmoles/g creatinine, roughly corresponding to some 2 percent of the mean intake of tyrosine and phenylalanine. In 19 out of 44 first determinations (43%) serum tyrosine was higher than 5 mg/100 ml. The highest excretion was 80 mmoles/g creatinine (case 4, Table IV), corresponding to about 35 percent of dietary tyrosine and phenylalanine. In this patient and also in her twin brother (case 1, Table IV) a considerable part of dietary tyrosine and phenylalanine was not utilized. The question arises whether the protein-intake should be diminished in such patients to a level at which no or little waste occurs. Then, however, we may risk introducing deficiencies of other amino acids. In this connection it may be mentioned that no excessive renal losses of other amino acids were observed in the prematures studied.

The incidence of tyrosyluria and tyrosinemia as found in our prematures roughly agrees with the results of Mathews and Partington [14], although an exact comparison is not possible due to differences in protein load and definition of tyrosinemia and tyrosyluria.

In 14 out of 50 patients published by Bremer et al. [1] serum tyrosine exceeded 10 mg/100 ml. In 13 out of the 44 of our prematures this was the case (Table VIII). La Du et al. [7] observed serum tyrosine concentrations

higher than 16 mg/100 ml in 7 out of 30 patients; we in 10 out of 44. But we could not confirm that in most prematures at a protein-intake lower than 5 g/kg/day serum tyrosine does not exceed 5 mg/100 ml as was stated by Hsia et al. [41] and La Du et al. [7].

From the time course study of tyrosyluria in our prematures (Figs 1 and 2) it can be concluded that normalization occurs rather suddenly and unpredictably within the first 8 weeks after birth. This points to an induction of the enzyme *p*-OHPPA-hydroxylase, caused by a mechanism hitherto unknown. Excess of ascorbic acid, 100 mg/kg/day, did not influence the excretory level of *p*-OHPLA and *p*-OHPPA, nor the start and rate of normalization, nor fasting serum tyrosine (Fig. 3). Therefore, addition of extra vitamin C to the diet of healthy prematures, who are not deficient in this vitamin, is not useful in view of the regulation of tyrosine metabolism. Avery et al. [10] however, reported that vitamin C, 60 mg/day, will "not always" prevent tyrosinemia at a protein load of 6 g/kg/day. Our results are in complete contradiction with those of Light et al. [9] who observed complete normalization of the patients on a protein load of 5 g/kg/day when 100 mg vitamin C was given daily. Similar observations were made by others [3,8]. No explanation is available for this discrepancy. Differences in protein load, deficiency in ascorbic acid and wrong interpretations of spontaneous disappearance of the abnormality all may contribute to the confusion.

From single determinations we could not establish an inverse correlation between tyrosinemia and tyrosyluria on the one hand and birth weight or gestational age on the other. However, in children with a delayed intra-uterine development the incidence of these abnormalities was higher as prematurity was more pronounced. Also the time course curves, giving more information than single determinations, point to this direction. The sudden enzyme induction at an unpredictable time accounts for the fact that a close correlation with age and weight hardly can be expected.

It is natural that the question whether the accumulation of tyrosine and *p*-OHPPA is harmful to the young child has been raised many times. Chemical events are closely parallel to those in phenylketonuria. In that disease accumulated phenylalanine and phenylpyruvate may inhibit metabolic enzymes and membrane transport, which may be the base of the developmental defect.

Direct toxic effects such as lethargy, bad drinking, and obtuned motor activity have been reported [10,42,24]. Impaired mental development was observed in children with a birth weight exceeding 2000 g and with plasma tyrosine levels of 15 mg/100 ml and higher [27]. Martin et al. [43] found a normal development of children with transient neonatal tyrosinemia. In this connection we should keep in mind that there are also patients with a permanent tyrosinemia and tyrosyluria without having liver and renal disease [30—32,34].

In such patients presumably a primary *p*-OHPPA-hydroxylase deficiency is present. These patients are all mentally retarded. Therefore it seems possible that a protracted exposure to high levels of tyrosine and *p*-OHPPA is harmful to the developing brain, which implies that dietary treatment should be considered in such patients. Also laboratory follow-up of tyrosinemia and tyrosyluria seems necessary in order to assess that the abnormalities, when present, are really transient.

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