Diagnosis and Treatment of Tyrosinosis

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This paper describes the diagnosis of tyrosinosis in a girl aged 10 months. Her subsequent progress after the institution of a diet low in phenylalanine and tyrosine has been good, and details of her treatment are discussed.

Tyrosinosis is an inherited metabolic disorder characterized by cirrhosis, severe hypophosphataemic rickets, renal tubular defects, and a derangement of tyrosine metabolism. The metabolic products arising from the deranged tyrosine metabolism point to a lack of p-hydroxyphenylpyruvate oxidase (Halvorsen, 1967).

Case History

The patient, the fifth child of unrelated parents, was born at full term after a normal pregnancy and labour. Her birthweight was 3400 g., and there was no history of neonatal jaundice. She was breast-fed for 9 months but was always fretful and difficult to feed. At the age of 3 months she had an *Esch. coli* urinary infection which responded well to treatment with nalidixic acid. At that time her blood urea was 35 mg./100 ml. and Hb 9.6 g./100 ml. Within the next 3 months she developed intermittent constipation with abdominal distension, and at the age of 6 months was backward in her motor activities. At 9 months she was found to have hepatosplenomegaly.

She had 3 older living male sibs, all of whom had eczema, 2 had intermittent abdominal pain, and 1 had migraine. Another male sib died at the age of 4 months, and necropsy showed that he had neonatal hepatitis, with intrahepatic cholestasis and moderate cirrhosis.

When she was admitted to this hospital at the age of 10 months she was a miserable baby with a poor appetite, constipation, and abdominal distension and discomfort. On examination, she was alert, below the third centile for height and weight, minimally jaundiced, slightly pigmented, and had an unusual facies. Her abdomen was very distended with the liver palpable 4 cm. below the right costal margin and the spleen palpable 1 cm. below the left costal margin.

Investigations. There were no abnormalities in Hb, white cell count, reticulocytes, platelets, or ESR.

A clean specimen of urine gave a deposit showing scanty pus cells, with a mixed growth on culture. X-rays showed changes of rickets in the knees and anterior ends of the ribs and femora. An intravenous pyelogram showed enlarged kidneys with prompt excretion of the dye; there was stretching of the calyces in the left and possibly in the right, which was suggestive of the adult type of polycystic disease. There was no clear sign of a focal lesion or paroxysmal features on the EEG.

Biochemical results are listed in Table I. With the exception of tyrosine, the plasma amino acid pattern was within the normal range, and in particular methionine was never raised (normal approx. 0.3 mg./100 ml. plasma). Tyrosine varied around 5 mg./100 ml. plasma (normal approx. 1 mg./100 ml.).

The urinary amino acid pattern (based on a creatinine standard) fluctuated somewhat before treatment was started. When first examined (age 11 months) there was a mild generalized aminoaciduria involving histidine, glycine, alanine, glutamine, and tyrosine. The same specimen examined by Professor Charles Dent using a total nitrogen standard gave a similar result. Further examination one month later then showed a gross aminoaciduria involving proline, lysine, asparagine, and threonine, as well as those amino acids previously identified, all of which were present in greater quantity. A variable excretion of tyramine was observed before the diet was instituted, but was thought to have arisen through bacterial contamination of the urine. The faecal amino acid pattern was normal and remained normal when examined after tyrosine and phenylalanine loads.

The absolute amounts of glucose, fructose, and galactose excreted in the urine varied throughout the day and from day to day, the fluctuations being primarily influenced by the dietary intake of carbohydrate. A disaccharide load indicated that absorption of sugars was normal and that galactose was metabolized better than fructose. A glucose tolerance test gave a normal glycaemic response.

All urine specimens tested gave a mauve coloration with ferric chloride and Phenistix, but no precipitate with 2:4 dinitrophenylhydrazine. Paper chromatography showed an abnormally high excretion of p-hydroxyphenyl-acetic and -lactic acids, together with N-acetyl tyrosine. Negligible quantities of p-hydroxymandelic acid were present.

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TABLE I
Biochemical Investigations

Investigations	Results
<i>Liver function</i>	
Plasma proteins (g./100 ml.)	5.5
Protein electrophoretic strip	Reduced albumin and raised γ -globulin
Serum transaminase (μ M pyruvate/100 ml. serum/hr.)	
SGOT	154 (normal 10-110)
SGPT	12 (normal 10-110)
Prothrombin time	10% of normal
Fasting blood sugar (mg./100 ml.)	True glucose = 40 Total reducing substance = 75
Glucose tolerance test	
Galactose-1-phosphate uridyl transferase	Normal
Caeruloplasmin	
Serum copper (mg./100 ml.)	70 (normal > 80)
Urine bilirubin	Not detected
Urine urobilinogen	Not detected
<i>Electrolytes</i>	
Sodium (mEq/l. plasma)	144
Potassium (mEq/l. plasma)	4.1
Chloride (mEq/l. plasma)	115
TCO ₂ (mEq/l. plasma)	10
Blood urea (mg./100 ml.)	12
<i>Calcium metabolism</i>	
Fasting calcium (mg./100 ml. plasma)	8.8 (normal 8.5-10.3) flame photometer
phosphorus (mg./100 ml. plasma)	1.6 (normal 4-5.5)
alkaline phosphatase (KA units/100 ml. plasma)	134 (normal 10-22)
Urinary calcium (mg./24 hr.)	18
Urinary phosphorus (mg./24 hr.)	154
<i>Renal function</i>	
Creatinine clearance	182 ml./min. per 1.73 sq. m.
Urinary protein (mg./24 hr.)	2.2
Osmolalities (mosm./kg.)	Random urines 572-220; maximum with fluid restriction 734
Urine pH	6.8 (when plasma T CO ₂ was 11mM/l.)
<i>Miscellaneous</i>	
Blood lead (μ g./100 ml.)	25
Urinary porphobilinogen	Negative

Amino acid loading tests. On the two occasions that the patient was given a tyrosine load (0.3 g./kg.) there was an increased excretion of p-hydroxyphenyl compounds during the subsequent 24 hours, but no p-hydroxyphenylpyruvic acid was detected in the freshly passed urine specimens, using 2:4 dinitrophenylhydrazine or ferric chloride reagents, or Phenistix.

The results of the phenylalanine loading tests are summarized in Table II and Fig. 1. Again, negative tests for aromatic keto acids were obtained. Paper chromatographic examination of the urine specimens showed that the excretion of Millon-positive substances paralleled the excretion of p-hydroxyphenyl-acetic and -lactic acids; no o-hydroxyphenyl-acetic acid was

FIG. 1.—Phenylalanine loading test. Data on phenylketonuric heterozygote, and normal subjects taken from Jervis (1960).

detected; only trace amounts of p-hydroxymandelic acid and N-acetyl tyrosine were present. In the six 4-hour urine specimens after the load, the excretion of p-hydroxyphenyl lactic was greater than that of p-hydroxyphenylacetic acid, while in the control periods the reverse held. Apart from an increase in the excretion of phenylalanine and tyrosine, no change in the urinary amino acid pattern was detected.

It was concluded that this child had an abnormality of tyrosine metabolism, which was associated with hepatocellular dysfunction, hypophosphataemic rickets, and renal tubular acidosis.

Treatment and Progress. The patient received the following treatment.

Electrolytes. An initial daily intake of 15 ml. of one-sixth molar sodium lactate was gradually increased to 30 ml., and this dosage has been continued.

Vitamin D. At the age of 1 year the patient was given vitamin D two months before starting the diet, because she had severe rickets. She was at first given 3000 units daily for 10 days, then 20,000 units daily for 3 weeks. The dose was further increased to 40,000

TABLE II
Urinary Excretion of Aromatic Substances during Phenylalanine Load

Day	Control			*Load (13.4 mM Phe)		
Protein intake Phe + Tyr equivalent	19.1 g. 11.0 mM			17.0 g. 9.9 mM		
4-hour periods	Phenolic acids	Tyrosine	Phenylalanine	Phenolic acids	Tyrosine	Phenylalanine
1000-1400	0.15	0.48	0.057	0.24	0.29	2.15
1400-1800	0.16	0.52	0.062	0.44	0.37	0.87
1800-2200	0.07	0.19	0.021	0.40	0.85	0.54
2200-0200	0.06	0.13	0.012	0.45	0.71	0.33
0200-0600	0.04	0.13	0.009	0.25	0.57	0.07
0600-1000	0.06	0.18	0.027	0.20	0.28	0.03
Totals/24hr.	0.54	1.63	0.188	1.98	3.07	3.99

Total Million 2.17 mM/24 hr.

5.06 mM/24 hr.

" " as % of intake 20%

22%

Phe + Tyr + phenolic

acids as % of intake 22%

29%

*The phenylalanine load was 0.3 g./kg., equivalent to 2.55 g.

units daily 3 weeks before starting the diet, because there was no radiological evidence of healing of the rickets. After 2½ months on the diet, another x-ray of the wrist showed an increase in severity of the rachitic changes. The daily dose of vitamin D was then increased to 60,000 units, and within 2 months there was complete healing of the rickets on radiological evidence. One month after this the child sustained a fractured clavicle while on holiday: this healed normally with good callus formation. After 12 months on the diet she became hypercalcaemic (plasma Ca 11.1 mg./100 ml.). After 4 weeks without any vitamin D she was given 40,000 units daily, but within a further 4 weeks the plasma calcium had risen again from 9.9 mg. to 10.6 mg./100 ml. Since this time the child has not had any vitamin D and there has been no recurrence of radiological or biochemical evidence of rickets.

Vitamin K. The hypoprothrombinaemia has proved difficult to control in spite of oral vitamin K supplements and biochemical evidence of improvement of other liver function tests since the child was started on the diet. She has been treated with vitamin K 10 mg. twice daily, which has maintained the prothrombin time at 40-50%. As she had no overt evidence of bleeding it was not felt justified to subject her to frequent vitamin K injections in order to raise the prothrombin content further.

Diet. The intake of tyrosine and phenylalanine was restricted by using a diet low in protein to which a synthetic amino acid mixture was added. Details are given in Appendix II. The diet was adjusted regularly depending on the plasma levels of tyrosine and phenylalanine. These were initially estimated twice weekly, then weekly, and later fortnightly. Over the first 3 months the child did not take the diet well: it proved difficult to control and the plasma tyrosine fluctuated between 1 and 5 mg./100 ml. Subsequently the dietary regimen became more stable, though the serum

branched-chain amino acids have remained low. It is interesting that 2 months after starting the diet when the plasma tyrosine was persistently high, there was biochemical evidence of increased hepatocellular damage. At the age of 2½ years the serum transaminases were normal and the plasma tyrosine varied from 1.3 mg., according to intake.

However, the branched-chain amino acids have fallen below normal values, in spite of subsequent increased intake of leucine, isoleucine, and valine.

During treatment the urinary amino acid pattern slowly improved, and became normal 14 weeks after starting the diet. It has remained either normal or weaker than normal, but δ-aminolaevulinic acid has been present in all urine specimens examined. The phenolic acid pattern in the urine also became normal, and has remained so, except when dietary indiscretion or fever produced raised plasma tyrosine and urinary phenolic metabolites.

The period on the diet has been marked by an outstanding increase in general well-being, motor development, and gain in height and weight. The child has now reached the 10th centile for height and the 50th centile for weight (see Fig. 2, 3, and 4). The liver has remained palpable 3 finger breadths below the right costal margin.

Discussion

In this patient the results from the phenylalanine loading test showed that the conversion of phenylalanine to tyrosine was considerably impaired, and that the formation of phenylpyruvic acid was virtually nil (compare Jervis, 1960). The former observation recalls the finding of increased plasma phenylalanine in transient neonatal hyper-tyrosinaemia (Hsia, Berman, and Slatys, 1964). Furthermore, the subsequent metabolism of tyrosine was considerably delayed, presumably due to the absence of p-hydroxyphenylpyruvate oxidase.

FIG. 2.—Weight chart showing response to diet and vitamin D.

This block could be only partial, since some 25% of ingested phenylalanine and tyrosine was recovered as urinary phenylalanine and Millon-positive substances (for technical reasons phenyllactic acid and phenylacetyl glutamine were not measured). Alternatively, some p-hydroxyphenylpyruvic acid might have been oxidized by renal p-hydroxyphenylpyruvate oxidase (La Du, 1967). The absence of significant amounts of p-hydroxyphenylpyruvic acid and its decomposition product p-hydroxymandelic acid (Gjessing, 1966) is surprising. A more sensitive paper technique (Smith, 1967) might

have revealed smaller amounts. This observation might indicate a deficiency of tyrosine- α -ketoglutarate-amino-transferase, so that slowly-formed p-hydroxyphenylpyruvic acid was rapidly converted to the corresponding lactic and acetic acids (compare Kogut, Shaw, and Donnell, 1967). Since phenylpyruvic acid was not detected during the phenylalanine loading test, the absence of o-hydroxyphenylacetic acid does not disprove the hypothesis that the enzyme p-hydroxyphenylpyruvate oxidase also converts phenylpyruvic acid to o-hydroxyphenylacetic acid (La Du, 1967).

FIG. 3.—Growth chart showing response to diet and vitamin D.

No explanation can be offered for the continued presence of δ -aminolaevulinic acid, which has been found in the urine of some patients with lead poisoning, nor for the low branched-chain amino acids in the plasma.

It is only recently that dietary treatment of tyrosinosis has become successful. Previous reports (Halvorsen, 1967; Aronsson *et al.*, 1966; Gentz *et al.*, 1967; Sass-Kortsak *et al.*, 1967) have shown an increase in growth and general well-being, and improvement in renal tubular and hepatic function similar to that seen in our patient.

We feel that dietary treatment of tyrosinosis is so successful that it should now become routine as for other inborn errors of amino acid metabolism.

The clinical condition of the older child with the completely developed syndrome is clear, but the aim should be to diagnose the condition as early as possible. In our experience, simple screening tests on urine for p-hydroxyphenylpyruvic acid using Phenistix, or ferric chloride and 2:4-dinitrophenylhydrazine reagents, have proved unreliable. For example, fresh urine specimens from 3 patients with proven disorders of tyrosine metabolism gave

FIG. 4.—Patient before treatment aged 13 months (a), and after treatment for 22 months, aged 2 years 11 months (b).

negative colour reactions, but subsequent chromatographic analysis of the 2:4-dinitrophenylhydrazones isolated from these specimens showed a significant excess of the hydrazone corresponding to p-hydroxyphenylpyruvic acid. A procedure for the investigation of infants suspected of having a disturbance in the metabolism of tyrosine and methionine is given in Appendix I. Plasma values of tyrosine greater than 4 mg./100 ml. and of methionine greater than 2 mg./100 ml., associated with abnormal amounts of urinary p-hydroxyphenylactic, -acetic, and -pyruvic acids (and p-hydroxymandelic acid—a decomposition product of the last) should be regarded with suspicion, particularly if ascorbic acid and protein intake are normal. A high protein intake or a deficiency in ascorbic acid may produce a tyrosyluria (Wong, Lambert, and Komrower 1967). On the other hand, a low protein intake may result in minimal changes in plasma tyrosine and in urinary amino acids and phenolic acids, as was the case in our patient.

Such a scheme of chemical investigation will of course detect patients with the benign form of transient hypertyrosinaemia (Avery *et al.*, 1967;

Bloxam *et al.*, 1960; Hsia *et al.*, 1964; J. W. T. Seakins and R. S. Ersser, 1967, unpublished observations). At the moment, there is no test known to the authors which distinguishes between the benign transient disturbance in tyrosine metabolism and the asymptomatic form(s) of tyrosinosis, and the question of dietary treatment has to be considered in the light of the clinical evidence. The finding of a low plasma phosphorus or disordered liver function tests would be in support of the inherited disorder.

Summary

The successful treatment of a 1-year-old child with tyrosinosis is described. Details of the diet are given together with the clinical and biochemical response.

In addition to the presumed deficiency in p-hydroxyphenylpyruvate oxidase, a loading test showed that other aspects of the metabolism of phenylalanine and tyrosine were also defective.

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REFERENCES

- Aronsson, S., Englesson, G., Jagenburg, R., and Palmgren, B. (1966). Clinical effects of low-tyrosine low-phenylalanine diet in a case of tyrosinemia. In *Symposium on Tyrosinosis*, p. 101. Ed. by L. R. Gjessing. University of Oslo Press, Oslo.
- Avery, M. E., Clow, C. L., Menkes, J. H., Ramos, A., Scriver, C. R., Stern, L., and Wasserman, B. P. (1967). Transient tyrosinemia of the newborn: dietary and clinical aspects. *Pediatrics*, **39**, 378.
- Bloxam, H. R., Day, M. G., Gibbs, N. K., and Woolf, L. I. (1960). An inborn defect in the metabolism of tyrosine in infants on a normal diet. *Biochem. J.*, **77**, 320.
- Clayton, B. E., Moncrieff, A. A., Pampiglione, G., and Shepherd, J. (1966). Biochemical and EEG studies in phenylketonuric children during phenylalanine tolerance tests. *Arch. Dis. Childh.*, **41**, 267.
- Gentz, J., Lindblad, B., Lindstedt, S., Levy, L., Shasteen, W., and Zetterstrom, R. (1967). Dietary treatment in tyrosinemia (tyrosinosis). *Amer. J. Dis. Child.*, **113**, 31.
- Gjessing, L. R. (1966). Studies in aromatic pyruvic acid metabolism in Fölling's disease, tyrosinosis, and neuroblastoma. In *Symposium on Tyrosinosis*, p. 55. Ed. by L. R. Gjessing. University of Oslo Press, Oslo.
- Halvorsen, S. (1967). Dietary treatment of tyrosinosis. *Amer. J. Dis. Child.*, **113**, 38.
- Hsia, D. Y.-Y., Berman, J. L., and Slatis, H. M. (1964). Screening newborn infants for phenylketonuria. *J. Amer. med. Ass.*, **188**, 203.
- Jervis, G. A. (1960). Detection of heterozygotes for phenylketonuria. *Clin. chim. Acta*, **5**, 471.
- Kogut, M. D., Shaw, K. N., and Donnell, G. N. (1967). Tyrosinosis. *Amer. J. Dis. Child.*, **113**, 47.
- La Du, B. N. (1967). The enzymatic deficiency in tyrosinemia. *ibid.*, **113**, 54.
- McCaman, M. W., and Robins, E. (1962). Fluorimetric method for the determination of phenylalanine in serum. *J. Lab. clin. Med.*, **59**, 885.
- Medes, G. (1932). A new error of tyrosine metabolism: tyrosinosis. The intermediary metabolism of tyrosine and phenylalanine. *Biochem. J.*, **26**, 917.
- Menzies, I. S., and Seakins, J. W. T. (1968). Sugars. In *Chromatographic and Electrophoretic Techniques*, 3rd ed., Vol 1. Ed. by I. Smith. Heinemann, London.
- Sass-Kortsak, A., Ficici, S., Paunier, L., Kooh, S. W., Fraser, D., and Jackson, S. H. (1967). Observations on treatment in patients with tyrosyluria. *Canad. med. Ass. J.*, **97**, 1089.
- Seakins, J. W. T., and Ersser, R. S. (1967). Effects of amino acid loads on a healthy infant with the biochemical features of Hartnup disease. *Arch. Dis. Childh.*, **42**, 682.
- Smith, I. (1968). In *Chromatographic and Electrophoretic Techniques*, 3rd ed., Vol 1. Ed. by I. Smith. Heinemann, London.
- Smith, P. (1967). Paper chromatography of keto acid 2, 4-dinitrophenylhydrazones. *J. Chromatog.*, **30**, 273.
- Westall, R. G. (1963). Dietary treatment of a child with maple syrup urine disease (branched-chain ketoaciduria). *Arch. Dis. Childh.*, **38**, 485.
- Wong, P. W. K., Lambert, A. M., and Komrower, G. M. (1967). Tyrosinemia and tyrosyluria in infancy. *Develop. Med. Child. Neurol.*, **9**, 551.
- iodoplatinate reagent for methionine (Smith, 1968) or with ninhydrin. These were then compared visually with combined standards equivalent to 5 and 10 mg. tyrosine and phenylalanine per 100 ml. plasma; another standard equivalent to 5 and 10 mg. methionine per 100 ml. plasma was used. Visual comparison proved adequate for dietary control. Plasma phenylalanine was also determined by the fluorimetric method of McCaman and Robins (1962), modified for use with the Beckman Ratio Fluorimeter. This latter method was used also for quantitating urinary phenylalanine after isolation on Zeo Karb 225 (H⁺), during loading studies.
- Urinary phenolic acids were isolated by the method described in Smith (1968). Urinary 2:4-dinitrophenylhydrazones were chromatographed on silica gel loaded paper (Smith, 1967). Freshly passed urine specimens were tested for aromatic keto-acids with Phenistix, ferric chloride, and 2:4-dinitrophenylhydrazine, and for pH.
- The methods for urinary sugars, and the disaccharide loading tests are described by Menzies and Seakins (1968).
- Millon-positive substances in urine were determined by the method described by Medes (1932). Tyrosine was removed by shaking the urine specimen with dry Zeo Karb 225(H⁺).
- Amino acid loading test.** The amino acid load, dissolved in a fruit syrup, was given at 10 a.m. after an overnight fast; food was withheld until 2 p.m. Urines were collected in 4-hour periods, and, after testing with Phenistix, were frozen on solid carbon dioxide. Crystals of chlorbutol were placed in the urine collecting bag to reduce bacterial metabolism. During the phenylalanine load, blood was collected at intervals for amino acid determinations.

Appendix II

The restriction of tyrosine and phenylalanine required a synthetic protein substitute low in phenylalanine and tyrosine to give adequate amino acids for growth and development. The child was therefore given a synthetic amino acid mixture in the amount of 2 g./kg. per day. The quantities of branched-chain amino acids were increased several times during the early stages of the dietary treatment, because reduced levels were demonstrated in her plasma by chromatography. The final composition of the amino acid mixture is shown in Table III.

The dietary intake of this infant before treatment was low in protein, possibly accounting for her relatively low plasma tyrosine before starting the diet. This, together with our experience with the phenylalanine intake required in phenylketonuria, led us to give a natural protein intake initially of 1 g./kg. per day, which provided approximately 50 mg. each of tyrosine and phenylalanine/kg. per day. The natural protein intake was then adjusted frequently according to the plasma phenylalanine and tyrosine. If the tyrosine was 0-1 mg./100 ml. plasma, the diet was increased by

Appendix I

Biochemical techniques. Urinary and faecal amino acids were examined by the methods described by Seakins and Ersser (1967). Plasma amino acids were separated by one-way chromatography and quantitated by the methods described by Clayton *et al.* (1966). For screening purposes, the specimens were run in duplicate on separate sheets and developed either with

0.5 to 1 g. natural protein daily; if 2-3 mg. the diet was left unchanged, and if the value was 4 mg. or more, the natural protein intake was decreased by 0.5 to 1 g. protein daily.

Other nutrients had to be considered

TABLE III

Composition of Amino Acid Mixture when Patient Weighed 10 kg.

L-Amino acid	g./day
L-alanine	0.689
L-aspartic acid	1.739
L-glutamic acid	3.477
L-cystine	1.873
L-histidine	0.975
L-proline	1.013
L-serine	1.664
L-threonine	0.809
L-arginine	1.153
L-lysine HCl	0.795
L-tryptophan	0.344
L-methionine	0.360
Glycine	1.739
L-leucine	1.007
L-isoleucine	1.200
L-valine	0.840
Total	19.667g.
	approximately 2g./kg.

(a) *Minerals*: the synthetic amino acid mixture is deficient in minerals, and the patient was therefore given the complete mineral supplement described by Westall (1963).

(b) *Vitamins*: she received 3 tablets of Ketovite and 5 ml. Ketovite liquid daily (Paines & Byrnes Ltd.).

(c) *Calories*: a diet containing 8-10 g. natural protein daily in addition to the amino acids is deficient in calories. This was a particular problem with the patient, since she could not tolerate excess fat and she refused suitable foods such as fruit and vegetables. Also she could only tolerate small frequent feeds. The calories provided by the diet were maintained at 100/kg. daily by providing low protein bread made from wheat starch, Aminex low-protein rusks (Liga Co., 23, Saxby Street, Leicester) served as a cereal, SMA (John Wyeth & Brothers Ltd.) as a source of milk, since it is relatively low in protein (0.4 g. protein and 20 calories/fl. oz. or 30 ml.), sugar, fruit juices, and Hycal (Beecham Ltd.).

Typical daily intake at the age of 1 year 9 months is set out in Table A.

TABLE A

Typical Daily Intake at the Age of 1 Year 9 Months

	Calories	Protein (g.)
20 g. amino acid mixture mixed with water to a paste and flavoured with 20 g. drinking chocolate (Cadbury) and 4 teaspoons sugar. The mineral supplement was added to this.	80	20
140 ml. (5 oz.) SMA milk mixture	90	1.2
6 Aminex low protein Rusks	60	0
Exchanges of Heinz Baby Foods to give natural protein allowed (approximately 1½ tins)	100	2.0
Low protein bread (made from wheat starch and chemical rising agent (Pfizer)	300	0.7
Blackcurrant juice, Welfare orange juice, Hycal	150	4.5
Fruit (not banana or dried fruits)	80	0
Small quantity sugar or crushed Aminex rusks, etc.	30	0.1
	20	0
	112	0
+ vitamins	1022	28.5

This diet provided 425 mg. phenylalanine and 425 mg. tyrosine when she weighed 10 kg. The quantities were adjusted according to changes in body weight and plasma levels of phenylalanine and tyrosine.

Addendum

The following investigation was prompted by our experience with a patient to be described in a forthcoming paper (Harries *et al.*, 1969). The investigations were carried out after our paper had been completed.

For one day and for the first meal of the next day, the intake of phenylalanine and tyrosine was increased by doubling the intake of natural protein. Chromatography of the blood taken after the last test meal gave 1 mg. phenylalanine and 3 mg. tyrosine/100 ml. plasma. The phenolic acid pattern of the control urine specimen was normal but the specimens collected at the end of 24 hours and after the last test meal showed abnormal amounts of p-hydroxyphenyl-acetic and -lactic acids. These results were confirmed by gas liquid chromatography. The 2:4-dinitrophenyl-hydrazine test for phenylketones was equivocal, but significant amounts of p-hydroxyphenyl-pyruvic acid, as its 2:4-dinitrophenyl derivative, were demonstrated by chromatography in the test urine specimens but not in the control. It is concluded that the impairment in tyrosine metabolism is still present.

REFERENCE

Harries, J. T., Seakins, J. W. T., Ersser, R. S., and Lloyd, J. K. (1969). Dietary treatment of an infant with some features of tyrosinosis. *Arch. Dis. Childh.* In the press.