Tyrosinosis

A Study of 6 Cases

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The syndrome to be described includes liver cirrhosis, renal tubular defects with vitamin D-resistant rickets, and abnormal tyrosine metabolism with raised levels of the serum tyrosine and an increased excretion of tyrosine and its metabolites in the urine (tyrosyluria). The syndrome has been described under several headings, reflecting the many unsolved problems of aetiology and pathogenesis. The term tyrosinosis was first used by Medes (1932), and though the main symptom in her case was muscular hypotonia, many of the biochemical features were in keeping with the cases described here. The term tyrosinosis was accepted at an International Symposium on Tyrosinosis held in Oslo in June 1965 (Gjessing and Halvorsen, 1966).

Tyrosinosis is probably not a very rare disease. Several cases with a similar clinical picture and with some of the characteristic biochemical findings have been reported previously, as discussed by Woolf (1963) and Gentz, Jagenburg, and Zetterström (1965).

In the first case studied by Medes there were increased amounts of p-hydroxyphenylpyruvic acid (p-HPPA) in the urine, which was interpreted as indicating inactivity of the enzyme p-HPPA-oxidase (Fig. 1). Sakai and Kitagawa (1957) and Sakai, Kitagawa, and Yoshika (1959) produced supporting evidence for inactivity of this enzyme in the case of a child with liver cirrhosis and progressive hypophosphataemic rickets, who excreted large amounts of tyrosine and hydroxyphenols. The child died at the age of 2 years with liver cirrhosis and hepatoma, and there was accumulation of tyrosine in the liver, spleen, and serum. In a liver biopsy p-HPPA-oxidase was lacking. These authors termed the disease 'atypical tyrosinosis', to distinguish it from Medes's case, though time has shown this type to be in fact the more common.

Zetterström (1963) studied 7 children with tyrosinosis, all from a rather isolated area in the southwestern part of Sweden, and Gentz *et al.* (1965) have given details of these. In 2 of the 7 cases, they

studied liver biopsies and found the α -keto-glutarate transaminase to be normal, while no p-HPPA-oxidase was detectable.

In 1964 Halvorsen and Gjessing described a 2-year-old girl with tyrosinosis who was put on a diet low in both tyrosine and phenylalanine. Their study provided evidence that tyrosine and/or its metabolites were of fundamental importance in the pathogenesis of the renal tubular lesions. Taniguchi and Gjessing (1965) demonstrated a lack of p-HPPA-oxidase in the liver and the kidney of one of our patients, and thus confirmed the findings reported by Sakai *et al.* (1959) and Gentz *et al.* (1965).

The purpose of the present report is to describe the clinical, biochemical, and morbid anatomical findings in 6 further cases, and to give a description of the natural course of the disease. A therapeutic trial of a low-tyrosine/low-phenylalanine diet is described and the findings discussed in relation to the pathogenesis of the disease.

Case Reports

The cases were 6 children from 3 different families, all living in the central part of southern Norway. No consanguinity has been found between the parents or between the different families, nor has any relationship been found between these cases and the reported Swedish families.

Family I. The parents were healthy, with 2 children, both affected.

Case 1a (M.S.). A girl, born May 26, 1962, birth weight 4,030 g., no icterus, developed normally until the age of 6 months when abdominal distension, with some diarrhoea and vomiting were noticed. At 8 months she was admitted to a hospital with pneumonia. Considerable hepatosplenomegaly was then found as well as active rickets, enlarged kidneys, a proconvertin-prothrombin (PP) index constantly below 9%, in spite of vitamin K injections, and a thrombocytopenia (132,000-49,000/c.mm.). There was no icterus and no petechiae.

She had slight hypertelorism, blue eyes, and blonde hair. Now and then she exuded a faint, peculiar sweet smell.

FIG. 1.—Scheme of tyrosine metabolism.

Benedict's test was slightly positive in the urine, but there was no glucosuria. The galactose-1-P-uridylyltransferase content of the erythrocytes was normal. Paper chromatography revealed a generalized aminoaciduria, particularly of tyrosine and glycine.

Her pneumonia responded to antibiotics, but large doses (200,000 IU) of vitamin D improved her rickets only slightly. At the age of 1 year she was transferred to the Children's Hospital, Rikshospitalet, Oslo.

She was then in fairly good condition. Height 75 cm. (50th centile), weight 8.9 kg. (10th centile), was mentally alert, and sat well, but could not stand or walk. The muscles were hypotonic. Radiography confirmed the clinical signs of rickets and also showed osteoporosis and slightly retarded bone-age (Fig. 2).

The liver was palpable 5 cm. below the costal margin. Laparotomy (August 23, 1963) revealed an enlarged firm liver with a nodular surface. The spleen and kidneys were also enlarged. Biopsy of the liver showed cirrhosis with marked nodular regeneration. The amino acid chromatogram from liver biopsy tissue showed 2-3 times higher concentrations of methionine than of tyrosine, but the patient was at that time very ill and had poor liver function.

The main laboratory findings are set out in Table I. The PP index was very low. The levels of the individual coagulation factors are shown in Table II: these were lower than usual with even advanced liver failure, and were in contrast to other liver function tests. The bromsulphalein retention test was zero, the serum protein level was 5.0 g./100 ml., with a normal electrophoretic distribution. The serum lipids were slightly raised, 1,170 mg., later 810 mg./100 ml. The total cholesterol was 206-166 mg./100 ml.

The stools were bulky, and between 4.5 and 19 g. fat was excreted per day. The ECG, EEG, CSF, bone-

marrow picture, and sweat electrolytes were all normal. The NPN was 20 mg. and the creatinine 1.0 mg./100 ml.

There was a slight hyperchloraemic acidosis, the total bicarbonate being 16, chloride 119, sodium 148, potassium 3.6 mEq/l. Blood pH was 7.38, the PCO₂ 23 mm. Hg.

FIG. 2.—Case 1a. Radiograph of the wrist (A) before, and (B) 8 months after the introduction of a low-phenylalanine/low-tyrosine diet.

TABLE I
Main Laboratory Findings

Case No.	1a	1b	2a	2b	3a	3b
Age (mth.)	12	3½	5	3½	4½	5-5½
Blood group	A Rh -		A Rh +	A Rh +		AB Rh +
<i>Blood</i>						
Hb (g./100 ml.)	10.0	10.6	7.2	10.2-6.4	10.4	10.1
Sedimentation rate (Westergren) (1 hr.)	11 mm.	2 mm.	6 mm.	3 mm.	3 mm.	2-6 mm.
Leucocytes per c.mm.	7,600	10,000	18,600	14,200	5,100	13,600
Thrombocytes (× 1,000) c.mm.	52-111		123			93
PP index (%)	5-12		< 5	< 5		< 5
Phosphorus (mg./100 ml.)	1.75-2.5					2.6, 2.4, 2.2
Alkaline phosphatase (Bodansky units)	38-24					47, 52, 49
Bilirubin (mg./100 ml.)	'Normal'		c. 2.2	1.8		2.1-3.0
<i>Urine</i>						
Protein	+			+		-
Blood	+					-
Benedict	+			+		+
Clinistix (glucose)	-			-		-
Ferric chloride	+			+		
2,4-DNPH	++			++		
Renal epithelial cells and casts	++			++		++

TABLE II
Coagulation Factors

Case	1a	3b
Age (mth.)	14	5½ 6
PP-index (%)	10	< 5
Quick time (%)	29	51
Cephalin time (%)	70	86
Proconvertin (%)	8	< 5
Proaccelerin (%)	64	16
Prothrombin (%)	30	< 5
Stuart factor (%)	40	—
AH A (%)	—	> 100
AH B (%)	22	< 5
Fibrinogen (mg./100 ml.)	—	125

FIG. 3.—Case 1a. Blood sugar response to glucagon injection (0.05 mg./kg.) at four different dates: 1, September 1, 1963; 2, 3, May 2 and 3, 1964; 4, June 4, 1965. Dietary treatment was from July 1964.

The fasting blood sugar level was normal, as was the response to an oral loading with galactose. Oral loading with glucose gave a slow but definite rise in glucose level (from 60 to 127 mg./100 ml.) with the maximum after 2 hours. There was no certain response to injections of glucagon (Fig. 3) or adrenaline.

The caeruloplasmin content in blood was low, 1.5-2.2 mg./100 ml. (normal 13-36). The copper excretion in the urine was normal, 30 µg./day (normal range 15-80), as was the copper content of the biopsied liver 283 µg./100 g. wet liver (normal 120-900).

Paper chromatography showed that the serum tyrosine was high (a quantitative measure was introduced later), while the other amino acids, especially methionine, were normal.

The urine contained small amounts of protein and an increased number of renal epithelial cells, casts, leucocytes, and erythrocytes, but no bacteria. Benedict's test was slightly positive, and paper chromatography revealed small amounts of galactose, fructose, and lactose. The ferric chloride test and the 2, 4-dinitrophenyl-hydrazine and Millon's reactions were all positive. Paper chromatography showed an aminoaciduria, especially for tyrosine and glycine, but with marked increase also of alanine, histidine, serine, glutamine, asparagine, and phenylalanine; methionine, however, was only slightly raised, and valine, leucine, taurine, cystine, lysine, and ethanolamine were normal. There was an excessive excretion of phenolic acids particularly p-HPLA (Table III). Following reduction of the phenylhydrazones of the keto acids to amino acids, tyrosine dominated but alanine, glutamic acid, and glycine were also found on one-way chromatograms.

The general condition of the patient was still fairly good. The high doses of vitamin D before admission to this hospital had temporarily raised the originally low levels of serum phosphorus and alkaline phosphatase. When she was put on a daily dosage of 3,000 IU vitamin D per day the levels again fell (Table IV).

TABLE III

Urinary Excretion of Phenolic Acid Amines and Amino Acids ($\mu\text{g./mg. creatinine}$), and Levels of Tyrosine and Methionine in Serum and Ascites (mg./100 ml.)

Family	I			II		III	
Children	Case 1a		Case 1b	Case 2a	Case 2b	Case 3a	Case 3b
Treatment	Before	After					
p-hydroxyphenyl-pyruvic acid ..	+++ +†	0	++++ +†		++++ +†		
p-hydroxyphenyl-lactic acid ..	5,200	10	++++ +†		5,500		
p-hydroxyphenyl-acetic acid* ..	1,200	22			830		
p-hydroxyphenyl-glycolic acid*	200	3			200		
Vanillyl-lactic acid ..	1.5	0			2		
Vanillyl-acetic acid ..	10	22			16		
Vanillyl-glycolic acid ..	4	6			3		
o-hydroxy-phenyl-acetic acid ..	1	1			1		
Tyramine ..	Normal	Normal					
Octopamine ..	0	0					
Normetanephrine ..	0.3	0.5					
Metanephrine ..	0.05	0.1					
Tyrosine ..	1,000		++++ +†		1,500		++++ +†
Methionine ..					400		
Serum tyrosine ..	7.5	1.5	3.0				
Serum methionine ..	0.6		5.0				
Ascites tyrosine ..			2.3				
Ascites methionine ..			6.0				

* Decomposition products of p-HPPA due to alkaline chromatography.

† Large excretion. ‡ Vanillyl 3-methoxy-4-hydroxy-phenyl.

TABLE IV

Case 1a: Laboratory Findings Before and After Dietary Treatment*

	1963			1964						1965			
	Feb.	June	Nov.	Apr.	May	July	Aug.	Sept.	Dec.	March	May	June	Sept.
Serum tyrosine (mg./100 ml.) ..						7.5	2.5	4.0		2.5	3.5	1.5	1.5
Serum phosphorus (mg./100 ml.) ..	1.75	2.5	1.2	1.4		1.8	2.4	3.4	4.7	2.9	3.4	4.5	4.2
Serum alk. phosphatase (Bodansky units) ..		38		85		104		77.5	23	25	41	38	17
Tubular reabsorption of phosphorus (TRP) ..		79		7	10	61	77		79	56		91	
PP index (%) ..	5	12	16	20		23		28	28		41	48	56
Thymol turbidity test ..		0.15	0.03	0.01				0.05					
SGOT (units) ..	100	79	80			73		70	50	53	73	48	40
Serum protein (g./100 ml.) ..	5.0		5.9	6.1		5.9		6.1	5.9	6.3	5.9	5.6	6.2
Thrombocytes, thousands/c.mm. ..	52	111		60		116		186	113	176	147	175	
Hb (g./100 ml.) ..		10.0	9.7	10.2		9.9		10.8	11.8	12.2	12.3		12.0

* From July 1964 a diet low in phenylalanine and tyrosine was given.

When the diagnosis of tyrosinosis was made, she was readmitted at the age of 23 months for a 4-week trial (May, 1964) with a low-tyrosine/low-phenylalanine diet (Halvorsen and Gjessing, 1964). Before the start of this diet her serum tyrosine level was 7 mg./100 ml. Following the diet the serum tyrosine fell towards normal levels, except for one occasion when she was given some milk in error, and the level rose to 6 mg./100 ml.

After 4 weeks on this diet the tubular reabsorption of phosphorus (TRP) increased from 7% to normal (96%), and the serum phosphorus from 1.4 to 3 mg./100 ml., while the alkaline phosphatase level was lower. The total amino nitrogen excretion did not decrease signifi-

cantly, but the amino acid chromatograms became more normal, except for conspicuous spots of tryptophan and methionine, due to the addition of DL-tryptophan and DL-methionine to the diet (Cymogran without added tyrosine). The phenolic acid excretion decreased to normal level, as shown in Fig. 4, which also shows the response to a tyrosine load. The ferric chloride and 2,4-dinitrophenylhydrazine tests became negative. When the phenylhydrazones of the keto acids were reduced to amino acids, tyrosine and alanine had disappeared, while glutamic acid and glycine were still present in small amounts. Addition of L-tyrosine to the diet reversed these improved findings, and when she was

FIG. 4.—Case 1a. Effect of diet and tyrosine load on urinary tyrosine, p-HPLA, and p-HPAA.

put on a regular diet, the values returned to their pre-treatment abnormal levels.

Four weeks after the end of this trial she was put permanently on the tyrosine-phenylalanine-restricted diet, which again induced the same set of biochemical changes. She has now (September, 1965) been on this diet for more than a year. Most of that time she has been at home, and the serum tyrosine level has remained between 1.5 and 4.0 mg./100 ml., i.e. slightly above normal. This is probably due to inefficient control of the tyrosine and phenylalanine intake, since under hospital control the serum tyrosine fell to 1.5 mg./100 ml.

After introduction of the diet there was a growth spurt (Fig. 5). The radiological signs of rickets disappeared completely without any increase in the vitamin D dosage of 3,000 IU per day (Fig. 2). Benedict's test of the urine became negative, and there was no excess of cells and casts. The liver, spleen, and kidneys decreased somewhat in size. The blood-sugar curve after glucagon injection returned to normal (Fig. 3). The improvement of liver function tests started shortly before the introduction of the diet and cannot be entirely attributed to this.

An interesting finding is that the low caeruloplasmin content increased to a normal level of 16.5 mg. after one year on the diet (3 years old). The stools are normal. She is energetic and mentally normal. She takes the

diet remarkably well, considering its lack of taste and its late introduction (at 23 months).

Case 1b (K.S.). A girl, born September 11, 1963, was breast-fed and developed normally until 3½ months old, when she lost her appetite and the stools changed, becoming more frequent, loose, and with some mucus. Two weeks later she became suddenly ill, and was brought to a local hospital. The examination there revealed a 4-month-old girl, well nourished, length 64 cm. (75th centile), weight 6,200 g. (25th centile). She was extremely ill, dyspnoeic, and with a distended abdomen. The liver was enlarged 8 cm. below the costal margin, the spleen was also enlarged. 160 ml. ascites were removed.

She had signs of rickets with pronounced craniotabes. Radiograph of the abdomen showed ileus with considerable dilatation of the intestines and many fluid levels.

The laboratory findings are tabulated in Table I.

Paper chromatography revealed a generalized amino-aciduria with a prominent tyrosine spot. The phenolic acid excretion was increased (Table III). The ascitic fluid revealed a high concentration of tyrosine and a 2-3 times higher concentration of methionine. A blood sample also showed 2-3 times larger amounts of methionine than of tyrosine.

She was treated with intravenous fluids, oxygen,

FIG. 5.—Case 1a. Growth chart showing a spurt after introduction of the low-phenylalanine/low-tyrosine diet.

antibiotics, and steroids, but died 16 hours after admission.

Necropsy revealed enlarged liver (190 g.), spleen (46 g.), and kidneys (56 and 62 g.). (Details of the liver, kidneys, and brain are given after the individual case reports.)

Both in the liver and in the kidney the free methionine level was 2-3 times larger than the tyrosine level.

The bones showed pronounced rickets. The other organs were normal, and there were no signs of intestinal obstruction nor of any infection.

Family II. The father had diabetes, the mother had always been healthy. They had 3 children, the two youngest being affected.

Case 2a (S.E.). A girl, born August 25, 1962, birth weight 5,000 g., with normal development up to the age of 5 months, became dyspnoeic, with symptoms of a mild upper respiratory infection, and was admitted to a local hospital.

Examination showed a pale infant, with hypotonic muscles. Radiography of the chest showed normal lung fields, but signs of rickets in the ribs.

The most prominent sign was a greatly distended abdomen, the peristaltic sounds being normal. The liver was firm and palpable 5 cm. below the costal margin. The spleen was also enlarged 2 cm. below the costal margin. The few laboratory tests done are tabulated in Table I. The PP test remained below 5%

in spite of vitamin K injections. The bleeding time was more than 10 minutes. The urine contained no protein or glucose.

She became jaundiced and died the second day after admission. No necropsy was made.

This case report does not by itself justify a diagnosis of tyrosinosis. It is only because her brother, born 2 years later, had tyrosinosis, that this diagnosis can in retrospect be presumed.

Case 2b (G.E.). A boy, born January 1, 1964, birth weight 3,550 g., developed normally, and nothing abnormal was noticed when he was routinely examined before inoculation when 3 months old, when urine Phenistix and Tes-Tape (glucose) tests were negative. From that time on, however, he failed to thrive. His parents noticed enlargement of his abdomen, he became easily dyspnoeic, and had a poor appetite. The stools gradually became loose and bulky. He was admitted to a local hospital when 3½ months old.

Examination revealed a rather pale, but well-nourished boy. Length 61 cm. (25th centile), weight 6,340 g. (75th centile). The abdomen was distended, with dilated veins (circumference 47 cm.). The liver was enlarged 2-4 cm. below the costal margin, and felt firm. There was a pronounced rickets with craniotabes and rosary. Radiological examination confirmed active rickets. The stools were large, pale, and fatty, and microscopical examination showed increased amounts of fat. There was a generalized enlargement of the lymph nodes. Biopsy of a node showed non-specific lymphadenitis

with some fibrosis. A bone-marrow aspirate showed a normal picture.

The main laboratory findings are tabulated in Table I. The NPN was 10 mg./100 ml. The PP test was below 5% on 8 occasions, in spite of vitamin K injections. The total serum protein was 5.0 g./100 ml. with normal electrophoretic distribution. The fasting blood sugar was 56 mg./100 ml. The thymol turbidity test was 0.24 and bilirubin 0.5-1.8 mg./100 ml.

The urine contained a small amount of protein, increased numbers of renal epithelial cells, and tubular casts. Benedict's test was slightly positive, but glucose was absent. The ferric chloride and Phenistix tests were positive, and the 2,4-DNPH test strongly positive.

Paper chromatography revealed a pronounced generalized aminoaciduria with a prominent tyrosine spot, and also a marked excess of glycine, histidine, serine, glutamine, asparagine, methionine, alanine, and phenylalanine, whereas the amounts of leucine, valine, cystine, and taurine were only moderately increased. The excretion of the phenolic acids was much increased as in Case 1a (Table III). After reduction of the phenylhydrazones of the keto acids to amino acids, tyrosine dominated, but alanine, glutamic acid, and glycine were also demonstrated on a one-way chromatogram, as in Case 1a.

The abdomen increased in size, ascites developed, and 850 ml. fluid was withdrawn (0.56 g. protein/100 ml.). A radiograph at that time showed greatly dilated intestinal loops. He died 2 weeks after admission.

Necropsy showed liver cirrhosis and changes in the kidneys and brain, which are described after the individual case reports.

Enzyme studies of the liver and kidney were carried out by Taniguchi and Gjessing (1965) and are reported elsewhere. They could find no activity of p-hydroxyphenylpyruvate oxidase in the liver or kidney, or of phenylpyruvate oxidase in the liver.

Family III. The parents were healthy; they have had 11 children. 3 died before the age of 7 months, one of them (T.O.S.) certainly, and one (A.S.) probably of tyrosinosis.

Case 3a (A.S.). A girl, born May 14, 1956, was a seventh child, birth weight 3,280 g. At about 3 months of age her abdomen became enlarged and she ceased to thrive. Her mother also noticed a peculiar, sweet smell from her. At 4½ months she became acutely ill with vomiting and was admitted to a local hospital, where she was found to have a distended abdomen with dilated veins. Liver and spleen were much enlarged. A radiograph showed dilated intestinal loops. Occult blood was present in the stools. She had no fever, and the white blood cell count was normal.

She was extremely ill and died before further investigations could be done. No necropsy was made.

From this case report alone no diagnosis can be made, but 4 years later a brother of this patient was born (Case 3b). The mother is convinced that they had the same disease, referring to the distended abdomen, the peculiar

sweet smell, and the failure to thrive. (She has also lost a third infant, a girl who died at home at 4 months from pneumonia. This girl did not show any of these signs.)

Case 3b (T.O.S.). A boy, born May 20, 1960, was the eleventh child in the family. He showed nothing abnormal during the first 4 weeks, but then began to vomit. His abdomen increased in size and the stools became large and foul smelling. He had some minor upper respiratory infections with otitis media. At 3 months he developed a seborrhoeic rash of moderate degree.

Because of the failure to thrive he was admitted to a local hospital, and 3 weeks later, when he was 5½ months old, was transferred to the Children's Hospital, Rikshospitalet, Oslo. Examination showed him to be short, length 61 cm. (2.5 centile), weight 6,040 g. (50th centile). He was slightly jaundiced (bilirubin 3 mg./100 ml.), but well nourished and in fairly good condition. The abdomen was enlarged with considerable hepatosplenomegaly and distended veins. Radiography showed greatly dilated intestinal loops. He had pronounced signs of rickets, confirmed by radiological examination and by serum analysis. The main laboratory findings are tabulated in Table I.

The PP-test was consistently below 5% in spite of vitamin K injections. The levels of different coagulation factors are shown in Table II. The thrombocytes were lowered (93,000/c.mm.), bone-marrow aspiration showed normal cell elements. Coombs' test was negative.

The SGOT was 61 units and thymol turbidity test 0.34. The total serum protein was 4.9-5.3 g./100 ml. with reduced albumin (2.6-2.2 g.) and increased globulin (1.3-2.0 g.).

The fasting blood sugar level was 82 mg./100 ml.

The galactose-1-P-uridyl-transferase content of the erythrocytes was normal. No cystine deposits were found. The spinal fluid was normal.

The urine gave a positive Benedict's reaction. Paper chromatography revealed the sugar to be galactose. There was no proteinuria. There were increased numbers of renal epithelial cells and casts. No inclusion bodies were found. Paper chromatography revealed a generalized aminoaciduria with a marked tyrosine spot.

NPN was 15-37 mg./100 ml. TRP showed no reabsorption of phosphorus on two occasions, and also a third time, after a 4-hour intravenous load with calcium.

The stools were pale, fatty, and bulky, and the fat excretion was 8.1 g. per day.

The nature of his disease was at this stage obscure. Initially he was thought to have galactosaemia and was given a galactose-free diet, but this was stopped after 2 weeks when the normal content of galactose-1-P-uridyl-transferase was known. He took his food fairly well, but continued to vomit and gained no weight. Addition of 3,000 IU vitamin D per day did not influence his rickets. The dosage was increased, but following a sudden gastro-intestinal haemorrhage he died.

Necropsy showed enlargement of liver (195 g.), spleen (37 g.), and kidneys (65 and 65 g.). The changes in the

liver, kidneys, and brain are described after the case reports.

The bones showed considerable rickets. The lungs showed moderate bronchopneumonia. The cause of death was diffuse haemorrhage from the gastroduodenal region.

Although serum tyrosine and phenolic acid excretion were not estimated in this case, the clinical picture, the aminoaciduria with a prominent tyrosine spot, and the morbid anatomical findings identical with proven cases of tyrosinosis, leave little doubt that this was a case of tyrosinosis.

Summary of Necropsy Findings

Necropsy was performed on Cases 1b (K.S.), 2b (G.E.), and 3b (T.O.S.). In all cases the *liver* was enlarged, the consistency being slightly firmer than usual, the colour was pale to yellowish-white, and the surface displayed numerous pin-head sized yellow nodules. The *kidneys* were slightly swollen and pale. The *brain* in Cases 1b and 3b was macroscopically normal, while in Case 2b the transverse diameter of the hemispheres was abnormally narrow.

Histology

Liver. The normal pattern was replaced by irregular groups of liver cells arranged in short cords or forming small acini. The nuclei varied slightly in size, but showed no signs of malignancy. The cytoplasm contained fine eosinophilic granules and sometimes vacuoles. Scattered regenerating lobules corresponded to the yellowish nodules, and in some of them the cells were filled with large vacuoles. Frozen sections stained with Sudan III showed the vacuoles filled with neutral fat. PAS staining was negative in the vacuoles but revealed traces of positive material in the cytoplasm of some of the acinar liver cells, and here and there also within the acini. Between the parenchymal cells there was an increase of fibrous tissue with scattered lymphocytes. There was a moderate proliferation of bile-ducts. The biopsy of the liver from Case 1a (M.S.) showed the same findings.

Kidneys. The glomeruli were well preserved. The convoluted tubules as well as the straight parts of the proximal tubules showed swollen, possibly degenerative changes of the epithelium. The convoluted tubules were distended and contained abundant eosinophilic material, often appearing in droplets or globules.

Pancreas. This was available for microscopical examination in Case 1b only. The islets of Langerhans were generally enlarged and increased in number. The acinar tissue seemed to be scarce, and there was some inflammatory infiltration of mononuclear cells in the fatty peri-pancreatic tissue.

Brain. There was probably some reduction in the number of neurones in the cerebral cortex, and others displayed a shrunken or poorly preserved nucleus. In the basal ganglia, corpus striatum, brain-stem, and cerebellum, the neurones were better preserved. The

astroglial cells had greatly enlarged, rounded, often empty-looking nuclei (Fig. 6), most prominent in the cortex and corpus striatum. Their cytoplasm was not visible, and granules as described in Alzheimer's type II cells were absent. They were PAS negative.

The white matter in Case 2b was poorly developed, and in Case 3b some tiny sudanophilic granules were present in the cytoplasm of the glial cells.

FIG. 6.—Case 1b. The astroglial nuclei in a section from the corpus striatum are seen enlarged, vesicular, and empty looking. (Gallocyanin-eosin. $\times 320$.)

Discussion

Clinical course. There seem to be two different patterns, (1) an acute, rapid course with death from liver failure within the first 6-8 months of life, or (2) a more prolonged course with death usually occurring during the first decade. Both clinical courses may appear in the same family indicating that it is more a question of degree than of different entities.

The familial nature of the disease is obvious. Gentz *et al.* (1965) described 7 patients from 4 families with a total of 13 sibs, and in our series there have been 6 cases from 3 families with a total of 16 sibs. It is likely that tyrosinosis is an autosomal, recessively inherited disease.

All our patients except one (Case 1a) ran an acute course. (A summary of the cases is given in Table V.) The histories were very similar in all 5, the first symptoms appearing in the gastro-intestinal tract with vomiting and diarrhoea. This was followed by a failure to thrive, distension of the abdomen, and a tendency for upper respiratory

TABLE V
Summary of the Cases

Case No. and Year of Birth	Sex and Age at Initial Symptoms	Main Symptoms and Signs	Outcome	Pathological Findings
1a 1962	F 6 mth.	Vomiting, abdominal distension, peculiar smell; muscular hypotonia, rickets; large liver, spleen, and kidneys	Living 3 yr.	Liver cirrhosis; nodular hyperplasia (biopsy)
1b 1963	F 3 mth.	Vomiting, diarrhoea; abdominal distension; haemorrhages; rickets; hepatosplenomegaly; ascites, paralytic ileus	Died 4 mth.	Liver cirrhosis; rickets; renal tubular changes; cerebral changes
2a 1962	F 5 mth.	Upper respiratory infections; epistaxis; abdominal distension; rickets; hepatomegaly	Died 5½ mth.	Not examined
2b 1963	M 3 mth.	Abdominal distension; failure to thrive; dyspnoea; rickets; lymphadenopathy; haemorrhages; hepatosplenomegaly, ascites	Died 4 mth.	Liver cirrhosis; renal tubular changes; cerebral changes
3a 1956	F 4½ mth.	Abdominal distension; failure to thrive; peculiar smell; haemorrhages	Died 5 mth.	Not examined
3b 1960	M 1 mth.	Upper respiratory infections; failure to thrive; peculiar smell; vomiting; diarrhoea; dermatitis; haemorrhages; rickets; hepatosplenomegaly; abdominal distension ascites	Died 6½ mth.	Liver cirrhosis; renal tubular changes; rickets; cerebral changes

infections. In 2 of the 5 acute cases the mother noticed a peculiar sweet smell from the infant.

Within a few weeks or months after the onset of symptoms, the condition deteriorated rapidly, ascites appeared and there was a tendency for bleeding with melaena, haematemesis, or haematuria.

On examination the infants usually looked well-fed, but had pronounced hepatosplenomegaly, often enlarged kidneys, and, even in infants below 6 months of age, rickets.

The laboratory studies usually revealed a moderate, normochromic anaemia, a normal sedimentation rate, and a moderate decrease of thrombocytes. The prothrombin-proconvertin-index (PP) was very low, and resistant to vitamin K injections in the cases examined. The serum bilirubin was only slightly raised until late in the disease.

In the acute cases the underlying renal and hepatic changes may be overlooked. Our 5 cases have been seen in 3 different hospitals, and the first impression has been that of an acute abdominal ailment. Plain x-ray films have shown dilated intestines suggesting a paralytic ileus. Perhaps this is caused by a hypotassaemia. One of the cases described by Fritzell, Jagenburg, and Schnürer (1964) had a decreasing level of potassium towards death.

In the cases with a subacute or chronic course,

either the renal tubular defects (corresponding to a Fanconi syndrome), or the hepatomegaly with signs of liver cirrhosis have been the presenting symptoms. The renal tubular defects seem to progress with age, giving an increasing excretion of phosphorus, amino acids, potassium, sugars, and protein, with renal epithelial cells and casts. These cases usually present at the age of 1-3 years on account of deforming, hypophosphataemic, vitamin D-resistant rickets.

A moderate thrombocytopenia is a frequent finding, both in the acute and in the prolonged cases. Whether this is due to splenic inhibition, or is related to the abnormal metabolism, is unknown. The number of platelets became normal following splenectomy in Case III of Gentz *et al.* (1965). Thrombocytopenia is also seen in other conditions with disturbed amino acid metabolism and acidosis such as hyperglycinaemia.

In contrast to the progressive renal changes the hepatic function seems rather to improve with age. Thus the raised thymol turbidity test, the transaminases, and the lowered PP index improved in one of the cases of Fritzell *et al.* (1964), in some of the cases of Gentz *et al.* (1965), and in our Case 1a. In the oldest case described by Gentz *et al.* (a 19-year-old boy), there were only doubtful signs of liver dysfunction, a finding that may link these cases to the original case described by Medes (1932).

Histological findings. The changes in liver and kidney in our cases were in accordance with earlier descriptions, without, however, any signs of malignancy. In all cases examined there was a marked nodular regeneration, and it is possible that this regenerative process may be a factor in the development into malignant hepatoma reported by others (Sakai *et al.*, 1959; Lelong, Alagille, Gentil, Colin, Le Tan, and Gabilan, 1963).

Gentz *et al.* (1965) described moderate mental retardation in some patients. They compared the clinical picture to that encountered in Wilson's disease, but no description of histological findings in the brain was given. The glial changes found in our cases correspond to what is found in prolonged hepatic diseases, and sometimes described as 'liver glia'. In none of the cases had this resulted in complete softening of any part of the brain. The deposit of the sudanophilic material in the white matter in Case 3b might indicate an impairment of the metabolic turnover, and the poor development of the white substance in Case 2b, indicated by the narrow hemispheres with apparently normal thickness of the cortex, might support this impression.

Diagnosis of tyrosinosis. This should be suspected in any case of liver cirrhosis in infancy and childhood, especially when combined with renal tubular defects. Galactosaemia has been first suspected in many cases, but the normal galactose-1-P-uridyl transferase excludes this. Wilson's disease may be more difficult to exclude because caeruloplasmin and serum copper were low both in our Case 1a and in the case of Sass-Kortsak, Jackson, and Scriver (1964).

A copper analysis in a liver biopsy may be necessary to exclude this possibility if the hypertyrosinaemia and tyrosyluria are not convincing. Cystinosis also has renal tubular changes, but very rarely pronounced liver disease. The relation of tyrosinosis to cystinosis has been thoroughly discussed by Gentz *et al.* (1965).

Biochemical considerations. The main biochemical feature of the disease is the defect in tyrosine metabolism. The serum tyrosine is usually increased to about 6-12 mg./100 ml. In the acute stage the serum methionine was found to be raised by Fritzell *et al.* (1964), and we found 2-3 times larger amounts of methionine than of tyrosine in the blood, liver, kidney, and in the ascitic fluid of the dying girl, Case 1b. Her sister, however, had a raised level of tyrosine, but normal amounts of methionine in her serum, and survived. The other fatal cases reported by Sass-Kortsak *et al.* (1964),

Greenberg, Chase, Lovrien, Hurwitz, and Efron (1964), and Perry, Hardwick, Dixon, Dolman, and Hansen (1965) as 'hypermethioninaemia with tyrosyluria', as well as Baber's (1956) case of 'congenital cirrhosis of the liver with renal tubular defects' belong, we consider, to the acute stage of tyrosinosis.

The raised level of methionine in the blood, liver, kidney, and ascitic fluid in the acute stage may be due to an inhibition of the new methionine pathway suggested by Perry *et al.* (1965), namely across the keto-analogue of methionine, precipitated by excessive amounts of tyrosine and its metabolites. The p-HPPA might inhibit the metabolism of α -keto- γ -methiolbutyric acid.

In our cases, as in most of the other cases, p-hydroxyphenyl-lactic acid has been the main metabolite excreted, and the excretion of this metabolite seems to be the best indicator of the degree of derangement in tyrosine metabolism, if the total Millon-positive substances are not determined. p-hydroxyphenyl-pyruvic and -acetic acid are also excreted in excess, but to a lesser extent, though variations occur in individual patients. The finding of only p-hydroxyphenyl-pyruvic acid as the main metabolite by Medes in her case of tyrosinosis makes it difficult to group the present cases with her case, but the finding may be related to the individual variations mentioned.

The findings in the cases of Sakai *et al.* (1959), Fritzell *et al.* (1964), Lelong *et al.* (1963), Gentz *et al.* (1965), and our own, all point to a block in tyrosine degradation at the level of p-hydroxyphenyl-pyruvate oxidase, and this has been confirmed by Sakai *et al.* (1959), Gentz *et al.* (1965), and in one of our cases (Taniguchi and Gjessing, 1965). All these enzyme studies have, however, been performed when the patients were on a normal diet.

Gentz *et al.* discuss the possibility that their cases are different from the other cases described, based on studies of the total Millon-positive substances excreted in the urine compared to the total tyrosine intake. In our opinion these differences more probably represent different degrees of enzyme activities, whether this is due to a genetically determined complete (or incomplete) lack of the enzyme, or to varying degrees of enzyme inhibition. Variations of the enzyme activity seem to be more pronounced in this disorder than in other presumably similar diseases (phenylketonuria), a finding that might support the hypothesis that the primary defect is not a lack of enzyme, but another genetically determined defect with secondary inhibition of this particular enzyme. While this is a possibility, most of the available data accords best with the theory

that a lack of p-hydroxyphenylpyruvate oxidase is the primary defect in this disease.

The main keto acid excreted in the urine in our cases has been p-hydroxyphenylpyruvic acid. When the keto acids were studied (Cases 1a, 2b) by means of reduction of the phenylhydrazones to amino acids, increased amounts of alanine, glutamic acid, and glycine were found besides large amounts of tyrosine. Following the tyrosine-phenylalanine restricted diet, tyrosine and alanine were not detected while glutamic acid and glycine were still present in amounts above normal.

The calcium/phosphorus metabolism is seriously disturbed in the disorder. The tubular reabsorption of phosphorus (TRP) is much reduced. Although this may be due to hyperparathyroidism secondary to calcium malabsorption or hyperexcretion, it is noteworthy that none of the patients have had hypocalcaemia, and that intravenous infusion of calcium did not influence TRP in Case 3b. The return to normal of the aminoaciduria following the low phenylalanine-tyrosine diet also points to the renal tubules as the primary site of dysfunction in the series of mechanisms leading to hyperphosphaturia and hypophosphataemia. This suggests that the main cause of the hypophosphataemia is inhibition of phosphorus reabsorption in the renal tubules by tyrosine or its metabolites. This view has received some support from a study of tyrosine overfeeding in immature mice, in which marked changes were produced in the kidneys (Halvorsen, (1966).

Steatorrhoea was observed in all 3 that were investigated (Cases 1a, 2b, 3b). In previous cases the steatorrhoea has been thought to be secondary to the liver disease, but its pathogenesis requires further investigation, because it is possible that the same metabolites that inhibit tubular reabsorption, at least of amino acids, may also inhibit absorption from the gut, a phenomenon similar to the situation in Hartnup disease. In our Case 1a the amino acids excreted in the faeces were only slightly raised compared with the normal controls.

In our cases the disturbance in the coagulation mechanism has been one of the most prominent features (Table II). In Case 1a proconvertin was much reduced, while in Case 3b both proconvertin, prothrombin, and antihæmophilic factor B were much reduced. These latter studies were performed a few days before death, and it is likely that they reflect the over-all collapse of liver function at that time. The caeruloplasmin level was also low during the first year in our Case 1a, becoming normal after one year of dietary treatment.

The carbohydrate metabolism is severely affected

at least during the first years. Although we have not observed much fasting hypoglycaemia, as noted by Sass-Kortsak *et al.*, our Case 1a failed to show a rise in blood glucose with adrenaline or glucagon in the first year, while a slight response occurred when she was tested at the age of 2 years. At the age of 3, following one year of dietary treatment, the response to glucagon was normal (Fig. 3). The mechanism of this effect is also unknown. The finding of galactose, fructose, and lactose in the urine also illustrates the impairment in the carbohydrate metabolism. The reducing substances disappeared from the urine following the dietary treatment in Case 1a.

Treatment. The treatment of this disorder has previously only been symptomatic. In the chronic cases high doses of vitamin D have been necessary to correct the rickets, sometimes with failures; in 2 of the cases of Gentz *et al.* severe hypercalcaemic crisis occurred. Corticosteroid therapy was without effect in the case of Lelong *et al.* (1963). The tyrosine- and phenylalanine-restricted diet reported here and in previous papers (Halvorsen and Gjessing 1964; Halvorsen, 1966) seems to be the logical treatment, and has been successful both in our case and in a similar case reported by Aronsson, Engleson, Jagenburg, and Palmgren (1966). The improvement has been most obvious in renal and bone-marrow function. The effect of the diet on liver function is more difficult to evaluate, as a spontaneous improvement of this function seems to occur with age. Introduction of the diet at an earlier stage of the disease may throw light on the pathogenesis of the liver cirrhosis.

Summary

Six children with liver cirrhosis, renal tubular defects, vitamin D-resistant rickets, and abnormal tyrosine metabolism are described. The 6 children came from 3 different Norwegian families, 2 children being affected in each family.

Similar cases previously described as tyrosinaemia, hepatorenal dysfunction, or hypermethioninaemia were probable examples of the same metabolic disorder, tyrosinosis.

Five patients died in an acute stage of the disease. The enzyme p-hydroxyphenylpyruvate oxidase in liver and kidney was absent in one case, and hypermethioninaemia was present in another. One patient survived in a chronic stage, and from the age of 2 years was successfully treated with a low phenylalanine, low tyrosine diet.

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