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DEVELOPMENT OF BIOCHEMICAL PROCEDURES FOR THE DIAGNOSIS OF GENETIC DISORDERS

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ABSTRACT

Normal patterns of carbohydrates, amino acids, alpha keto-acids and mucopolysaccharides in the urine of newborns, infants and children (n = 118) were established. Comparison with those found in the case group (n = 429) that included cases of developmental delay, seizure disorders, mental retardation, autistic behavior, multiple congenital anomalies, multiple skeletal deformities, sepsis, meningitis, etc. highlighted significant findings.

The biochemical procedures done include the common screening procedures for phenyketones, α-keto acids, reducing sugars, tyrosine, cystine and mucopolysaccharides; one- and two- dimensional thin layer chromatography for identification of amino acids, ketoacids and sugars and cellulose acetate electrophoresis for identification of mucopolysaccharides.

In addition to glucose, several in the case group had other sugars like fructose, galactose, xylose and lactose in various combinations. There were twenty in the case group found to be positive for phenylketones, 24 for alphaketones and one positive for cystine. Various amino acid patterns: a generalized increase or an increase in specific amino acids (e.g. glycine, alanine, beta-alanine, methionine, proline, OH-proline, tyrosine, histidine, lysine, glutamate, leucine, isoleucine and valine) were also demonstrated.

The normal samples that were positive in the turbidity test for mucopolysaccharides were found to have chondroitin sulfates A and C by electrophoresis. However 48 in the case group were found to have urinary heparan sulfate and dermatan sulfate either singly or in combination in addition to chondroitin sulfates A and C.

The study established reference data on urinary metabolites in infants and children. It also documented the presence of inborn errors of metabolism among Filipino children.

INTRODUCTION

There is a discernible change in attitude towards genetic disorders amongst medical practitioners. The present attitude is founded on the hope that medicine can help the afflicted and that if the disorder is diagnosed early the undesirable consequences can be prevented. Much of this change in attitude can be traced to better

understanding of metabolism and its regulation and to the advances in molecular biology that has brought to light the promise of gene therapy. At present, replacement therapy, nutritional control, modulation therapy and surgical modification are but a few of the possible modalities in the treatment of genetic disorders.

In the Philippines, much work still needs to be done to generate information regarding the prevalence of genetic disorders and the magnitude of the health problems they have engendered. Although not all known genetic variations are associated with disease, many manifest with severe lifelong handicaps; frequently with mental retardation. Clow, et al, (2) indicated that about 6% of the admissions to pediatric hospitals in the US are due to disorders which are clearly genetic, while another 15% of the admissions are for conditions which are thought to have at least some genetic component. This situation is most probably true in our institution even as most of our cases present as infection or malnutrition.

This study intends to make inexpensive biochemical procedures accessible to clinicians and their patients for the diagnosis of various metabolic disorders in amino acid, sugar, and mucopolysaccharide metabolism. Laboratory procedures were adapted to local conditions and standardized to simplify interpretation. Normal patterns and qualitative test results were established for different age groups and hence can be used in the evaluation of test results in individual patients.

MATERIALS AND METHODS

Collection and Preparation of Urine Samples

Urine samples were obtained from clinically normal newborn infants in the PGH Nursery, and from infants and children being followed up in the PGH Pediatric Well-baby and Continuity Clinics from 1995 to 1996. Consent was obtained from the parent or guardian who was interviewed about past and present illnesses using a prepared questionnnaire (Appendix A). Infants and children with history of seizures or convulsions, developmental delay, liver, heart or kidney diseases were not included. Fresh urine was collected using a wee bag and kept in cold ice prior to processing. Samples were filtered, labeled and stored at -70°C until analysis.

Urine samples from the pediatric patients (case group) from the PGH Department of Pediatrics Wards and Outpatient department and private clinics who were referred to the laboratory for the period 1993 to 1996 were submitted by the parents/ guardians who were instructed to collect at least 20-30 ml urine which were kept in ice in transport to the laboratory. These samples were also filtered, labeled and stored at -70°C until analysis. Both random and early morning urine samples were accepted for analysis.

Materials and Reagents:

All reagents were analytical grade and purchased as follows: from Sigma Chemical Company (U.S.A.) - all amino acids, Azure A, alpha-keto glutaric acid, alpha ketobutyric acid, alpha-ketovaleric acid, alpha-ketoisocaproic acid, barium acetate, N-butanol, citric acid anhydrous, cetylpyridinium chloride, cetyltrimethyl ammonium bromide, chondroitin sulfate A, B and C, creatinine, Ferric chloride anhydrous, fructose, galactose, glucose, heparan sulfate, lactose, naphtho-resorcinol, 1-nitrosonaphthol, para-nitrophenylhydrazine, ninhydrin, oxaloacetic acid, *p*-hydroxyphenyl-pyruvic acid, phenylpyruvic acid, pyruvic acid, ribose, sodium chloride, sodium cyanide, sodium nitroprusside, xylose; from Ajax Chemical Company (Australia) - dimethylsulfoxide, absolute ethanol, formic acid, sodium carbonate anhydrous, n-butanol, formic acid; from J.T. Baker Chemical Corp. Company (Germany)-ammonium hydroxide, acetone, copper sulfate, sodium citrate, sodium hydroxide, glacial acetic acid, isopropanol, pyridine; from Merck - hydrochloric acid conc., ethyl acetate; from BDH Lab - conc. nitric acid; from Mallinkrodt - conc. sulfuric acid; from Fluka - ethyl formate, resorcinol; form H. Sargent & Co. - ligroin.

Test strips (Combus-9) for determination of pH and detection of the presence of protein, ketones, urobilinogen, bilirubin and blood in the urine were purchased from Boehringer Mannheim, Inc.

Thin layer chromatography of amino acids was done using Cellulose in aluminum sheets, 20 x 20 cm (Merck #5552) while silica gel 60 in aluminum sheets, 20 x 20 cm (Merck #5553) was used for reducing sugars and α-keto acids.

Biochemical Procedures

Test strips –

Preliminary determination of urinary pH and qualitative detection of protein, ketones, urobilinogen, bilirubin and blood were done on fresh urine samples using Combus-9 test strips.

Tests for Reducing Sugars –

Benedict's Test for reducing sugars was performed according to the method described by Henry (4). Samples that tested "positive" or "traces" were subjected to thin layer chromatography using silica gel as described by Schmidt (9) for identification.

Tests for amino acids and α-keto acids –

Nitrosonaphthol test for tyrosine by Perry, *et al.* (7), Nitroprusside test for cystine by Knox (5), Ferric chloride test for phenylketones as described by Cassidei, *et al.* (1), Dinitrophenylhydrazine test for a-keto acids as decribed by Penrose & Quastel (6) were done as qualitative screening tests.

One- and two-dimensional thin layer chromatography were done for definitive identification of amino acids and one-dimensional thin layer chromatograpy for α-keto acids.

Tests for Mucopolysaccharides –

The Cetyltrimethylammonium bromide Turbidity test as described by Renuart, et al, (8) was used to detect the presence of mucopolysaccharides which were further identified by electrophoresis in cellulose acetate using the method by Wessler and DiFerrante (13,3).

RESULTS AND DISCUSSION

There were a total of 118 infants and children in the normal group and 429 pediatric patients in the case group. Tables 1 and 2 show the distribution of the normal population and the case group into 4 age levels. Table 3 lists the different clinical presentations of

the pediatric patients - most of whom were referred for developmental delay, seizure disorder or mental retardation.

Visual examination of the urine samples, test strip and Benedict's test results from the normal infants and children are summarized in Table 4. There were several turbid urine samples and urine color ranged from light yellow to orange-brown. Acidity was from pH 5 to pH 8 with the newborns having a more acidic range of pH 5-6. Slight proteinuria (30 mg/dL) were noted in 12 of 23 newborn urine samples. Likewise, glucose was detected in 15 of 23 newborn samples. Two of the 23 newborn urine samples were positive for blood. Only one urine sample (1/33) was positive for protein, while another was positive for glucose in the 2-6 yrs group. A single sample from the 6-12 years group tested 2+ for bilirubin. Except for one other urine sample in the 6-12 yrs group that was positive for protein, all the rest tested negative for the other substances. Ketones and urobilinogen were not detected in all urine samples.

The Benedict's test in the newborn group correlated with the glucose test strip results but additionally gave positive results in 15 among the infant group,18 in the 2-6 yr group and another 18 in the 6-12 yr group. The reducing sugars were later identified as glucose by thin layer chromatography (Figure 1).

In thin layer chromatography (TLC), the different monosaccharides were distinguished from each other by their different mobilities except glucose and fructose because of similarity in mobilities (Figure 1). The Seliwanoff's test for ketoses done for confirmation of the presence of fructose was negative in all the samples from the normal group, thus establishing the presence of glucose alone.

It was noted that several urine samples which tested "negative" in Benedict's showed glucose bands in thin layer chromatography. This underlines the microsensitivity of the latter.

The presence of reducing sugar (glucose) in the urine of almost half of the normal group is indicative of inefficient reabsorption by the kidney at that level of development. Additionally, it may also reflect a high intake of simple sugars among Filipino children. The variable time of urine collection precludes any valid conclusions

regarding glucosuria because post-prandial urine may give a positive test for Benedict's.

The case group data on Benedict's test indicated "traces" and "positive" results in 145 of the 429 pediatric referrals (Table 5). Thin layer chromatography results (Table 6) showed that 64 of the 145 "positive" samples contained glucose alone. The rest were mixtures of other monosaccharides and lactose. The typical patterns of mixtures of monosaccharides in thin layer chromatography are illustrated in Figure 2. These are most evident in the patients referred for developmental delay or seizure disorder. Lactose was detected alone or in combination with monosaccharides in 31/429 of the pediatric patients.

All normal urine samples tested negative in the screening tests for phenylketones (Ferric Chloride test), keto acids (DNPH test), tyrosine (Nitrosonaphthol test) and cystine (Nitroprusside test). The results are indicated in Table 7.

A detailed presentation of the results of the different screening test on amino acid metabolism on samples from the case group is given in Appendix B. A summary of the positive results alone is given in Table 8.

The Ferric chloride test for phenyl ketones was positive in 20 urine samples from cases presenting with mental retardation (9/16) autistic behavior or sepsis (7/25). This is an interesting finding as this test screens for phenylketonuria which has not been reported in the Philippines. Phenylketonuria is an inherited disorder that is the result of a failure of the liver enzyme phenylalanine hydroxylase to convert phenylalanine to tyrosine and is characterized biochemically by an excessive accumulation of phenylalanine and its metabolites (chiefly phenylpyruvic acid). However, phenylpyruvic acid was detected by thin layer chromatography only in one of the cases with autistic behavior. Thus, the positive Ferric chloride test could be due to other metabolites like α-ketoisocaproic acid, α-ketoisovaleric acid and α-ketoglutaric acid. This is borne out by thin layer chormatography results in Table 11 and illustrated in Figure 3.

Alpha-ketoisocaproic acid and alpha-ketoisovaleric acid are metabolites of branched chain amino acids leucine and valine respectively. Their detection in the urine implies an increase in blood

level and is usually observed in disorders in the metabolism of branched-chain amino acids whose principal manifestation is sepsis in the newborn and mental retardation in older children (11). Thiamine deficiency may also be implicated in these cases because of the important role of thiamine as coenzyme in the oxidative decarboxylation of α-keto acids.

Amino acids are a normal constituent in serum in urine and are detectable by one-dimensional and two-dimensional thin layer chromatography. *Aminoacidemia* is the presence in serum of one or more amino acids in quantities greater than normal or the presence of certain amino acids or intermediates of amino acid metabolism not usually found in serum (12). On the other hand, *aminoaciduria* is the urinary excretion of one or more amino acids in quantities greater than normal or the excretion of certain amino acids or intermediates of amino acid metabolism not usually found in the urine or both (12). It is good to remember that aminoaciduria may be due to increased levels in the blood or due to lack of active reabsorption in the kidney so that a generalized aminoaciduria may be secondary to tubular abnormalities.

Figures 4 and 5 show the typical patterns of amino acids in normal urine and in samples where a specific amino acid is increased or where there is a generalized increase in amino acids. Table 9 classifies the different patterns and amino acids found in the cases where there is an increase in a specific or few amino acids or a generalized increase. Very prominent is the number of cases of specific aminoaciduria in cases of developmental delay, seizure disorder, mental retardation and sepsis in the newborn.

Glycinuria was demonstrated in 14 cases (5 with developmental delay, 7 with a seizure disorder, 2 with meningitis and 1 with pneumonia). Glycinuria is usually implicated in pediatric nephrolithiasis. It could also be part of hypophosphatemic rickets in which case there will also be glucosuria and prolinuria. In cases of iminoglycinuria, glycine, proline and OH-proline are found elevated in the urine at the same time.

Other specific amino acids found increased in some of the cases are alanine in 3 cases, β-alanine in 1 case, methionine in 2 cases, tyrosine in 1 case, histidine in 2 cases and lysine in 7 cases. A follow-up of these cases is suggested for a better appreciation of the significance of these findings.

Generalized aminoaciduria is found in many disorders involving impairment of the renal tubules. Generalized aminoaciduria can also be demonstrated in the normal newborn, in hepatic necrosis, infectious disorders, malnutrition, in Vitamin C deficiency and in Vitamin D deficiency.

Cetyltrimethylammonium bromide is used for the detection of abnormal amounts of the acid mucopolysaccharides (hyaluronic acid, chondroitin sulfate A, chondroitin sulfate C, dermatan sulfate, heparin sulfate and keratan sulfate) in the urine. The procedure is based on the fact that cetyltrimethyammonium bromide yields water-insoluble quaternary salts with the sulfated and non-sulfated acidic mucopolysaccharides. The method results in the formation of turbidity and/or precipitate in the presence of excessive amounts of the mucopolysaccharides. The detection of these compounds in the urine helps in the definitive diagnosis of the mucopolysaccharidoses. They result from a deficiency of specific lysosomal enzymes involved in the degradation of heparan sulfate, dermatan sulfate and keratan sulfate either singly or in combination. The clinical features are derived from the involvement of multiple tissues and organs including the connective tissues, the central nervous and respiratory systems. The Morquio syndrome is characterized by keratansulfaturia, Sanfilippo syndrome by heparansulfaturia and Maroteaux-Lamy syndrome by dermatansulfaturia. Hurler's, Hunter's and Schie's syndromes are characterized by varying amounts of heparan and dermatan sulfate.

Heparan sulfate and dermatan sulfate together were demonstrated in 4 of the patients referred for developmental delay (Figure 6). This would be consistent for the Hunter-Hurler-Schie group of mucopolysaccharidoses. On the other hand, 16 patients with developmental delay, 5 with seizure disorder, one with mental retardation, 6 with autistic behavior, 2 with multiple skeletal deformities, 2 with rickets and one with multiple congenital anomaly were noted as having heparan sulfate which indicates the consideration of Sanfilippo syndrome.

SUMMARY

This study brought about the standardization of biochemical procedures for the detection of metabolic disorders under local

conditions. In addition, normal patterns of carbohydrate, amino acid, α-keto acids and mucopolysaccharides in newborn, infants and children were established. In comparing normal patterns with those obtained from the case group, significant biochemical data relevant to developmental delay, seizure disorders, mental retardation and sepsis among pediatric patients (Tables 13, 14 and 15) were noted. There is a need to firm up the correlation between these biochemical data and clinical manifestations. More information can be obtained from follow-up studies on the development of these children.

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Table 1. **Age distribution of the normal study group consisting of newborns at the PGH Nursery and infants and children reporting to the PGH Well Baby Clinic in 1995-1996.**

AGE GROUP	MALE	FEMALE	TOTAL	%
0 - 28 days	15	8	23	19.4
29 days - 2 yrs	16	15	31	26.3
>2 yrs - 6 yrs	17	16	33	28.0
>6 yrs - 12 yrs	16	15	31	26.3
TOTAL	64	54	118	100.0

Table 2. **Age distribution of the case group consisting of patients from the PGH Department of Pediatrics and private clinics referred for metabolic screening at the Department of Biochemistry and Molecular Biology, UP College of Medicine, 1993-1996.**

AGE GROUP	MALE	FEMALE	TOTAL	%
0 - 28 days	32	16	48	11.2
29 days - 2 yrs	110	77	187	43.6
>2 yrs - 6 yrs	78	50	128	29.8
>6 yrs - 12 yrs	38	28	66	15.4
TOTAL	258	171	429	100.0

Table 3. Clinical description/presenting symptoms of patients in the case group, 1993-1996.

CLINICAL DESCRIPTION	MALE	FEMALE	TOTAL
Developmental Delay	70	46	116
Seizure disorder	49	42	91
Mental retardation	6	10	16
Metabolic Disorder Mucopolysaccharidoses 5 Hypothyroidism 2 Chronic Renal Disease 1	4	4	8
Autistic Behavior	18	4	22
Congenital Brain Anomaly	1	1	2
Multiple Skeletal deformities	1	1	2
Rickets	5	1	6
Sepsis	17	8	25
Meningitis	3	3	6
Pneumonia	6	3	9
Cyanosis	1	0	1
Hypotonia	0	2	2
Multiple Congenital anomaly	4	3	7
Congenital nephrotic syndrome	2	0	2
Request without clinical description	65	49	114
TOTAL	252	177	429

Table 4. Summary of results of test strips and the Benedict's test on urine samples from the normal pediatric study population, 1995-1996.

TESTS	AGE GROUP			
	0-28 days	29 days - 2 years	2 days - 6 years	6 days - 12 years
Number of samples	23	31	33	31
Nature and Color of urine	(clear) 8 (turbid) 15 - light yellow to brown	(clear) 17 (slightly turbid) 7 - very light yellow to yellow	(clear) 20 (slightly turbid) 7 - light yellow to orange	(clear) 25 (slightly turbid) 4 (turbid) 2 - light yellow to orange
Test Strips a) acidity b) proteins c) glucose d) ketones e) urobilinogen f) bilirubin g) blood	 pH 5-6 (-)11, (+)12 (-) 8, (+)15 (-) (-) (-) (-)21, (+)2	 pH 5-8 (-) (-) (-) (-) (-) (-)	 pH 5-8 (-)32, (+)1 (-)32, (+)1 (-) (-) (-) (-)	 pH 5-8 (-)30, (+)1 (-) (-) (-) (-)30, (++)1 (-)
Benedict's Test (-) clear blue to turbid green (traces) (+) green solution with orange precipitate	 9 1 13	 13 2 13	 15 18	 13 3 15

Table 5. Number of urine samples in the case group giving positive, traces and negative results in the Benedict's test, 1993-1996.

	BENEDICT'S TEST (Reducing Sugars)			TOTAL
CLINICAL DESCRIPTIONS	Clear Blue Solution (-)	Turbid green Solution (traces)	Orange Precipitate (+)	
DEVELOPMENTAL DELAY	82	8	26	116
SEIZURE DISORDERS	60	7	24	91
MENTAL RETARDATION	10	1	5	16
METABOLIC DISORDER	6	0	2	8
AUTISTIC BEHAVIOR	16	1	5	22
CONGENITAL BRAIN ANOMALY	1	0	1	2
MULTIPLE SKELETAL DEFORMITIES	1	0	1	2
RICKETS	5	0	1	6
SEPSIS	17	4	4	25
MENINGITIS	3	1	2	6
PNEUMONIA	5	2	2	9
CYANOSIS	1	0	0	1
HYPOTONIA	1	0	1	2
MULTIPLE CONGENITAL ANOMALY	5	0	2	7
CONGENITAL NEPHROTIC SYNDROME	1	0	1	2
Request w/o clinical description	1	2	6	9
TOTAL	285	46	99	429

Table 6. Reducing sugars detected in the urine of 144 pediatric patients positive for Benedict's test, by thin layer chromatography, 1993-1996.

(G - glucose, Ga - galactose, F - fructose, X - xylose, L - lactose)

	SUGARS DETECTED BY THIN LAYER CHROMATOGRAPHY								
CLINICAL DESCRIPTIONS	G	GF	GL	GX	GF L	GL Ga	GF XL	GF LGa	L
DEVELOPMENT DELAY	13	5	5	4	2	3	1		1
SEIZURE DISORDERS	12	2	6	3	4	2		1	1
MENTAL RETARDATION	4							1	1
METABOLIC DISORDER		1				1			
AUTISTIC BEHAVIOR	4		1	1					
CONGENITAL BRAIN ANOMALY				1					
MULTIPLE SKELETAL DEFORMITIES	1								
RICKETS								1	
SEPSIS	1	1	3	1	1				1
MENINGITIS	3								
PNEUMONIA	2		1		1				
CYANOSIS									
HYPOTONIA	1								
MULTIPLE CONGENITAL ANOMALIES	1								
CONG NEPHROTIC SYNDROME			1			1			
Requests without clinical description	22	3	8	2		8			
TOTAL	64	12	25	12	8	15	1	3	4

Table 7. Summary of the results of the qualitative tests for amino acids and alpha-keto acids on the urine of normal pediatric population, 1995-1996.

	FERRIC CHLORIDE (Phenylketones)	DNPH (α-keto acids)	NITROSO-NAPTHOL (Tyrosine)	NITRO-PRUSSIDE (Cystine)
AGE GROUPS	*greenish, greyish to green gun metal*	*yellow opalescent/ turbid orange/ppt within 5 mins*	*orange-red color within 2-5 minutes*	*deep purple which fades gradually*
0 - 28 days	(-) yellow to dark brown	(-) clear yellow to turbid orange	(-) clear light yellow to slightly turbid light yellow	(-) clear light yellow to very light cherry red
29 days to 2 years	(-) light yellow to yellowish brown	(-) clear yellow to golden yellow	(-) clear to turbid light yellow	(-) clear very light yellow to very light pink
2 years to 6 years	(-) light yellow to orange	(-) clear yellow to slightly turbid orange	(-) clear light yellow to turbid light yellow	(-) clear light yellow to pink
6 years to 12 years	(-) light yellow to brown	(-) clear yellow to turbid orange	(-) clear yellow	(-) clear light yellow to light pink

Table 8. Summary of the positive results in the screening tests on urine samples from the case group, Department of Biochemistry and Molecular Biology, 1993-1996.

	FERRIC CHLORIDE (Phenylketones)	DNPH (α-keto acids)	NITROSO-NAPTHOL (Tyrosine)	NITRO-PRUSSIDE (Cystine)
Positive Results	*greenish, greyish to green gun metal*	*yellow opalescent/ turbid orange/ppt within 5 mins*	*orange-red color within 2-5 minutes*	*deep purple which fades gradually*
SEIZURE DISORDERS (91)		(traces) 1		(+)1 *clear very light cherry red*
MENTAL RETARDATION (16)	(+)9 *gun metal green*	(+)9		
AUTISTIC BEHAVIOR (22)	(+)1 *turbid green*	(+)2 *turbid yellow orange*		
SEPSIS (25)	(+)7 *gun metal green*	(+)9 *turbid yellow*		
MENINGITIS (6)		(trace)1 *turbid orange*		
REQUESTS W/O CLINICAL DESCRIPTION	(+)3 *gun metal*	(+)2 *turbid yellow orange*		

Table 9. Amino acid profile of urine samples from the case group, Department of Biochemistry and Molecular Biology, 1993-1996.

	AMINO ACID PROFILE		
	Normal Pattern	Increase in a specific amino acid	Generalized increase in amino acids
DEVELOPMENTAL DELAY	91	10	15
SEIZURE DISORDERS	69	14	8
METABOLIC DISORDER	7	3	6
MENTAL RETARDATION	7	0	1
AUTISTIC BEHAVIOR	18	2	2
CONGENITAL BRAIN ANOMALY	1	0	1
MULTIPLE SKELETAL DEFORMITIES	0	0	2
RICKETS	3	0	3
SEPSIS	15	8	2
MENINGITIS	3	2	1
PNEUMONIA	4	1	4
CYANOSIS	1	0	0
HYPOTONIA	2	0	0
CONGENITAL NEPHROTIC SYNDROME	1	0	1
MULTIPLE CONGENITAL ANOMALY	5	0	2
Requests w/o clinical description	83	14	17
TOTAL	310	54	65

Table 10. Specific amino acids increased in the urine of patients from the case group, Department of Biochemistry and Molecular Biology, 1993-1996.

Amino Acid	Develop mental delay n = 116	Seizure Disorder n = 91	Mental retardation n = 16	Autistic behavior n = 22	Meningitis n = 6	Sepsis n = 25	Pneumonia n = 9
GLY	5	7			2		1
ALA	1	1	1				
β-ALA		1					
MET	1	1					
PRO	1	2					
OH-PRO		1					
TYR	1						
HIS		1		1			
LYS	1	3	2	1			
GLU						2	
LEU						6	
ILEU						6	
VAL						6	

Table 11. The type of α-keto acids detected by thin layer chromatography in the urine samples from the case group, Department of Biochemistry and Molecular Biology, 1993-1996.

	α-keto isocaproic acid	α-keto isovaleric acid	α-keto glutaric acid	Phenyl pyruvic acid	Pyruvic acid
DEVELOPMENTAL DELAY					
SEIZURE DISORDER			1		
MENTAL RETARDATION	4	4	5		
METABOLIC DISORDER					
AUTISTIC BEHAVIOR			1	1	1
CONGENITAL BRAIN ANOMALY					
MULTIPLE SKELETAL DEFORMITIES					
RICKETS					
SEPSIS	8	8	1		
MENINGITIS			1		
PNEUMONIA			1		
CYANOSIS					
HYPOTONIA					
MULTIPLE CONGENITAL ANOMALY					
CONGENITAL NEPHROTIC SYNDROME					
REQUESTS WITHOUT CLINICAL DESCRIPTION	1	1	2	3	2

Table 12. Summary of the results of the Cetyltrimethylammonium bromide turbidity test (CAB Turbidity test) on the urine samples from the case group, Department of Biochemistry and Molecular Biology, 1993-1996.

	CETYLTRIMETHYLAMMONIUM BROMIDE TEST			
	(+)	(Traces)	(-)	TOTAL
DEV'TL DELAY	23	3	90	116
SEIZURE DISORDERS	17	4	70	91
MENTAL RETARDATION	3	2	11	16
MET DISORDER	3	2	3	8
AUTISTIC BEHAVIOR	11	5	6	22
CON BRAIN ANOMALY	1	0	1	2
MUL SKEL'L DEFORM	2	0	0	2
RICKETS	2	0	4	6
SEPSIS	4	1	20	25
MENINGITIS	0	2	4	6
PNEUMONIA	3	1	5	9
CYANOSIS	1	0	0	1
HYPOTONIA	2	0	0	2
MULTIPLE CONGENITAL ANOMALY	1	0	6	7
CONGENITAL NEPHROTIC SYNDROME	0	0	2	2
w/o clinical description	2	1	6	9
TOTAL	100	33	296	429

Table 13. Electrophoretic identification of the mucopolysaccharides in the urine samples from the case group, Department of Biochemistry and Molecular Biology, 1993-1996.

	MUCOPOLYSACCHARIDES			
	Chondroitin sulfate A/C	DERMATAN SULFATE	HEPARAN SULFATE	KERATAN
DEV'TL DELAY	26	4	20	0
SEIZURE DISORDERS	21	0	5	0
MENTAL RETARDATION	5	0	1	0
MET DISORDER	5	1	5	1
AUTISTIC BEHAVIOR	16	0	6	0
CON BRAIN ANOMALY	1	0	0	0
MUL SKLTL DEFORM	2	0	2	2
RICKETS	2	0	2	0
SEPSIS	5	0	4	0
MENINGITIS	2	0	0	0
PNEUMONIA	4	0	2	0
CYANOSIS	1	0	0	0
HYPOTONIA	0	0	0	0
MULTIPLE CONGENITAL ANOMALY	1	0	1	0
CONGENITAL NEPHROTIC SYNDROME	0	0	0	0
Request w/o clinical description	2	0	0	0

Table 14. Summary of positive findings in the urine samples of 116 pediatric patients referred for Developmental Delay at the Department of Biochemistry and Molecular Biology, 1993-1996.

Reducing Sugars	Traces		8
	Positive		26
	Glucose	13	
	Glucose, Fructose	5	
	Glucose, Lactose	5	
	Glucose, Xylose	4	
	Glucose, Fructose, Lactose	2	
	Glucose, Lactose, Galactose	3	
	Glucose, Fructose, Lactose Xylose	1	
	Lactose	1	
Amino acids	Generalized increase in amino acids		15
	Increase in specific amino acid		10
	Glycine	5	
	Alanine	1	
	Methionine	1	
	Proline	1	
	Tyrosine	1	
	Lysine	1	
	Keto acids		0
Mucopolysaccharides	Traces		3
	Positive		23
	Chondroitin Sulfate A/C	26	
	Dermatan Dulfate	4	
	Heparan Sulfate	20	

Table 15. Summary of positive findings in 91 urine samples from the pediatric patients referred for Seizure Disorder at the Department of Biochemistry and Molecular Biology, 1993-1996.

Reducing Sugars	Traces		7
	Positive		24
	Glucose	12	
	Glucose, Fructose	2	
	Glucose, Lactose	6	
	Glucose, Xylose	3	
	Glucose, Fructose, Lactose	4	
	Glucose, Lactose, Galactose	2	
	Glucose, Fructose, Lactose Galactose	1	
	Lactose	1	
Amino acids	Generalized increase in amino acids		8
	Increase in specific amino acids		14
	Glycine	7	
	Alanine	1	
	β–Alanine	1	
	Methionine	1	
	Proline	1	
	OH-Proline	1	
	Histidine	1	
	Lysine	3	
	Cystine (Qualitative)	1	
	Keto acids (Qualitative)	Traces	1
	α-ketoglutaric acid	1	
Mucopolysaccharides	Traces		3
	Positive		23
	Chondroitin Sulfate A/C	26	
	Dermatan Dulfate	4	
	Heparan Sulfate	20	

Table 16. Summary of positive urinary findings in 16 pediatric patients referred for Mental retardation at the Department of Biochemistry and Molecular Biology, 1993-1996.

Reducing Sugars	Traces		1
	Positive		5
	Glucose	4	
	Glucose, Fructose, Lactose Galactose	1	
	Lactose	1	
Amino acids	Generalized increase in amino acids		6
	Increase in specific amino acids		3
	Alanine	1	
	Lysine	3	
	Phenylketones (Ferric Chloride)		9
	Keto acids (Qualitative)		9
	α-ketoglutaric acid	5	
	α-ketoisovaleric acid	4	
	α-ketoisocaproic acid	4	
Mucopolysaccharides	Traces		2
	Positive		3
	Chondroitin Sulfate A/C	5	
	Heparan Sulfate	1	

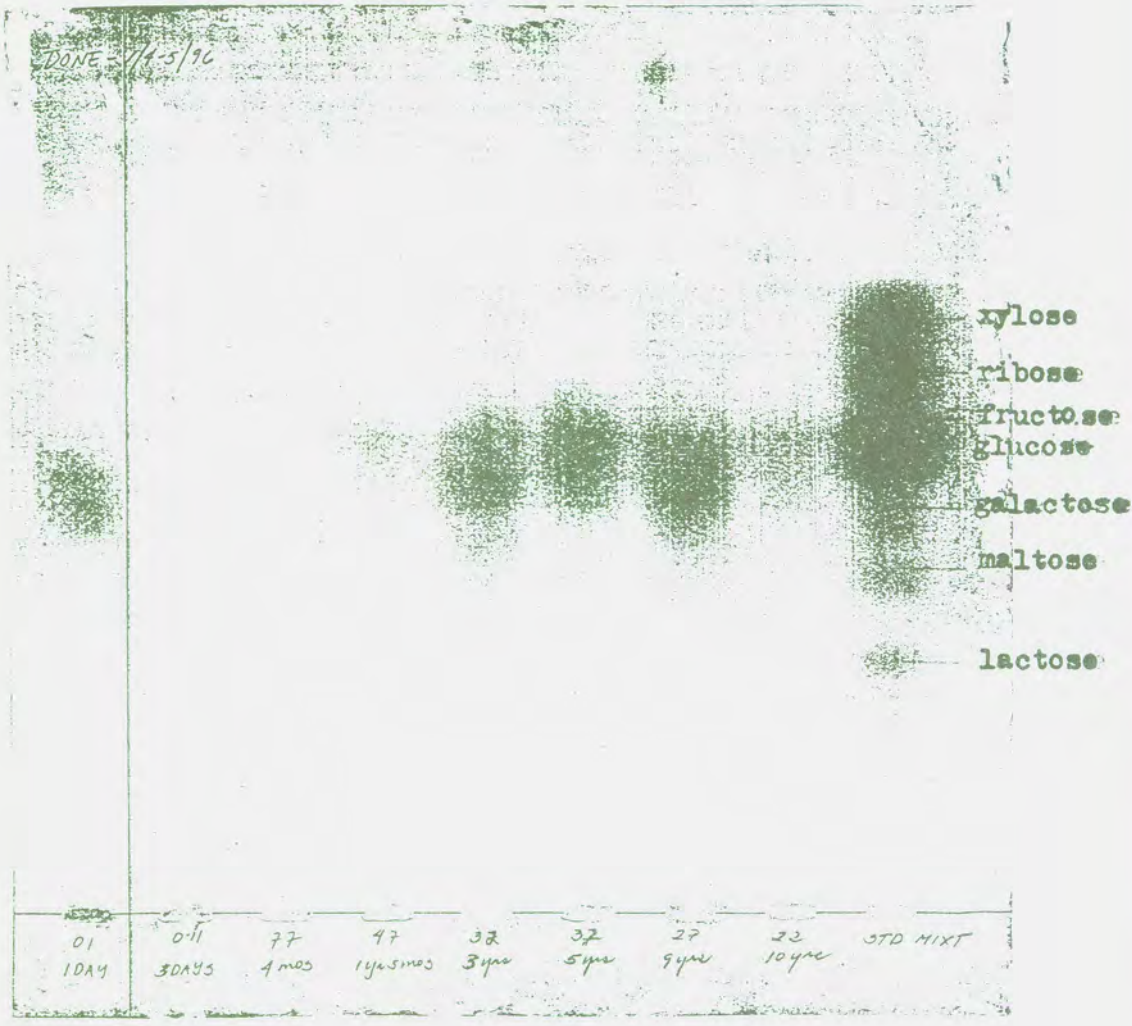


Figure 1. Thin Layer Chromatogram of a standard mixture of reducing sugars and of urine samples from the different pediatric age showing typical carbohydrate patterns.

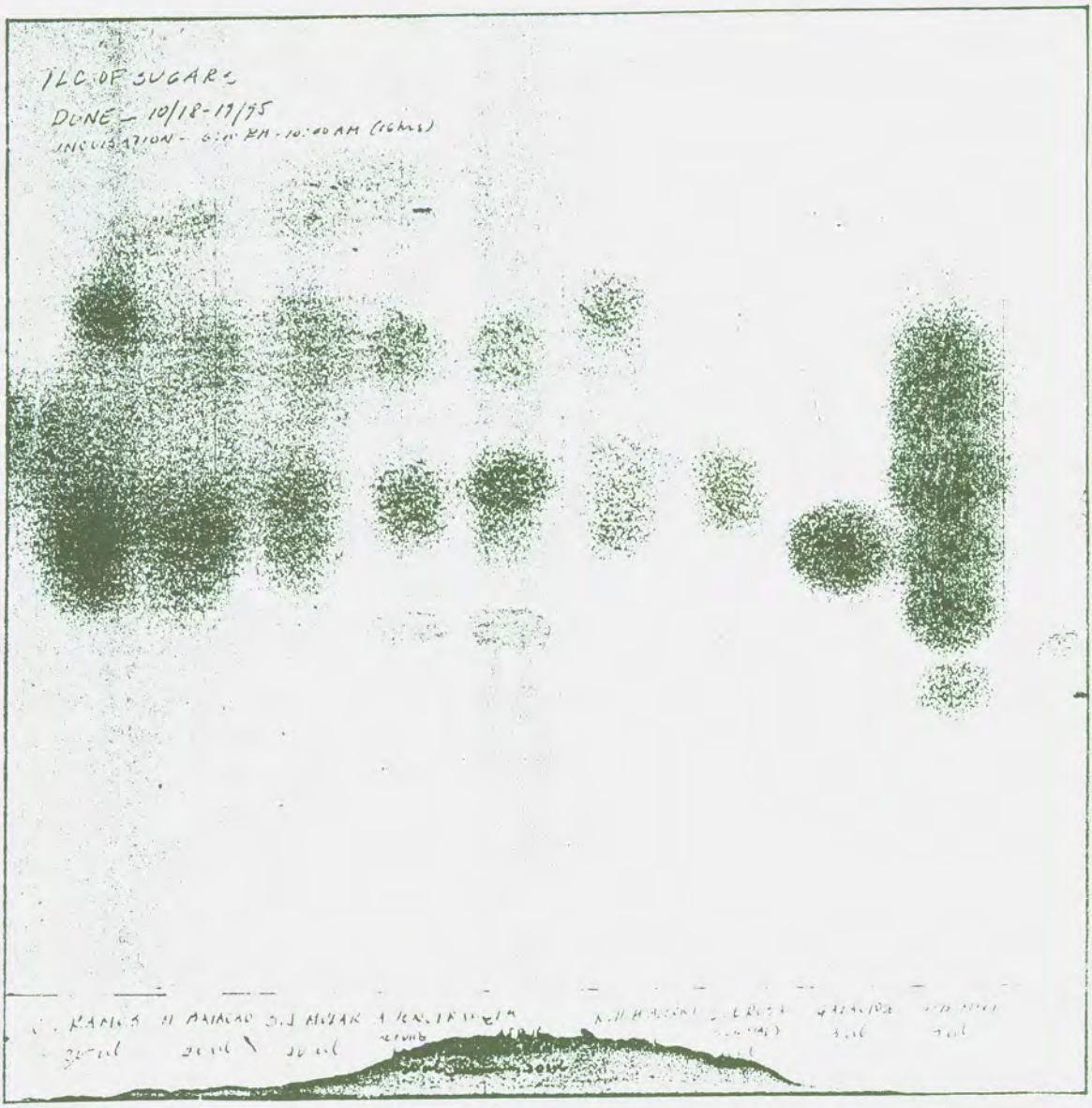


Figure 2. Thin Layer Chromatography of reducing sugars in the urine of some patients from the case group (1993-1996) compared with a standard mixture.

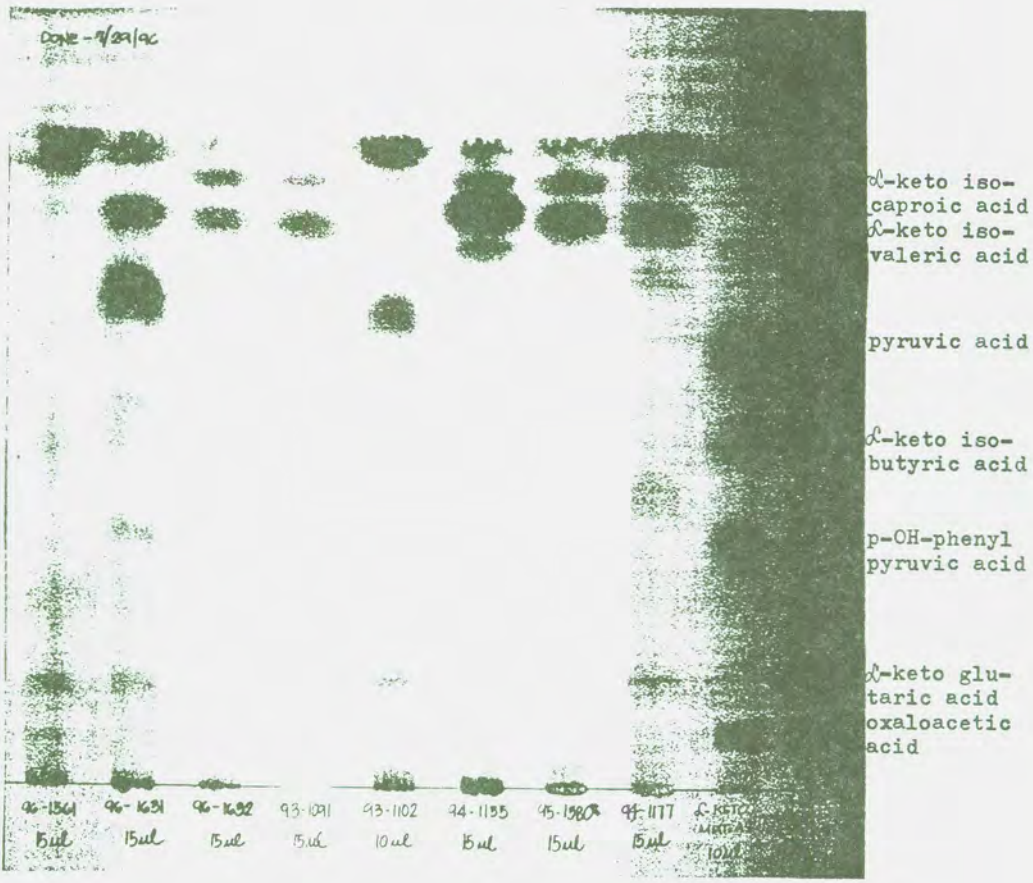


Figure 3. Thin Layer Chromatography of α-keto acids from a standard mixture and from selected urine samples of pediatric cases, 1993-1996.

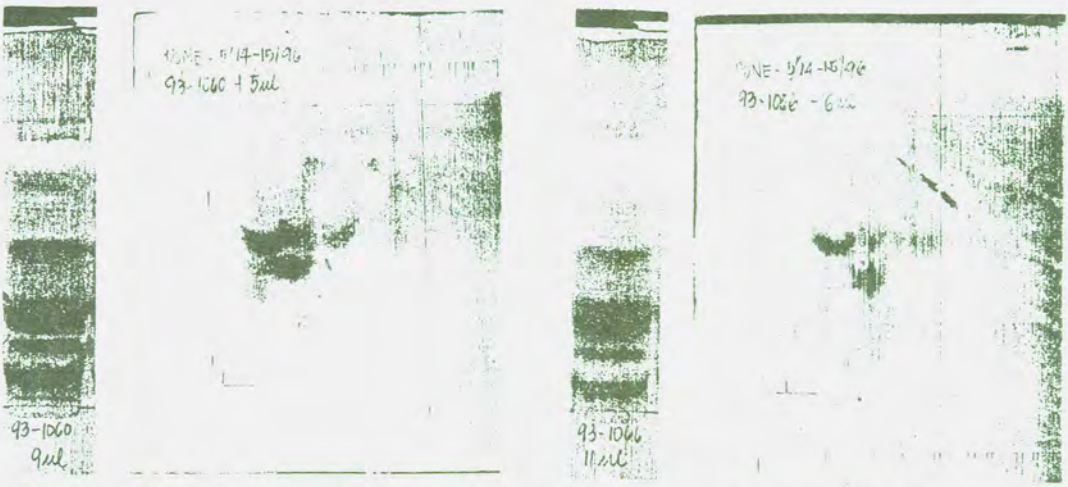


Figure 4. Typical one- and two-dimensional thin layer chromatograms of urinary amino acids from the normal group, 1995-1996.

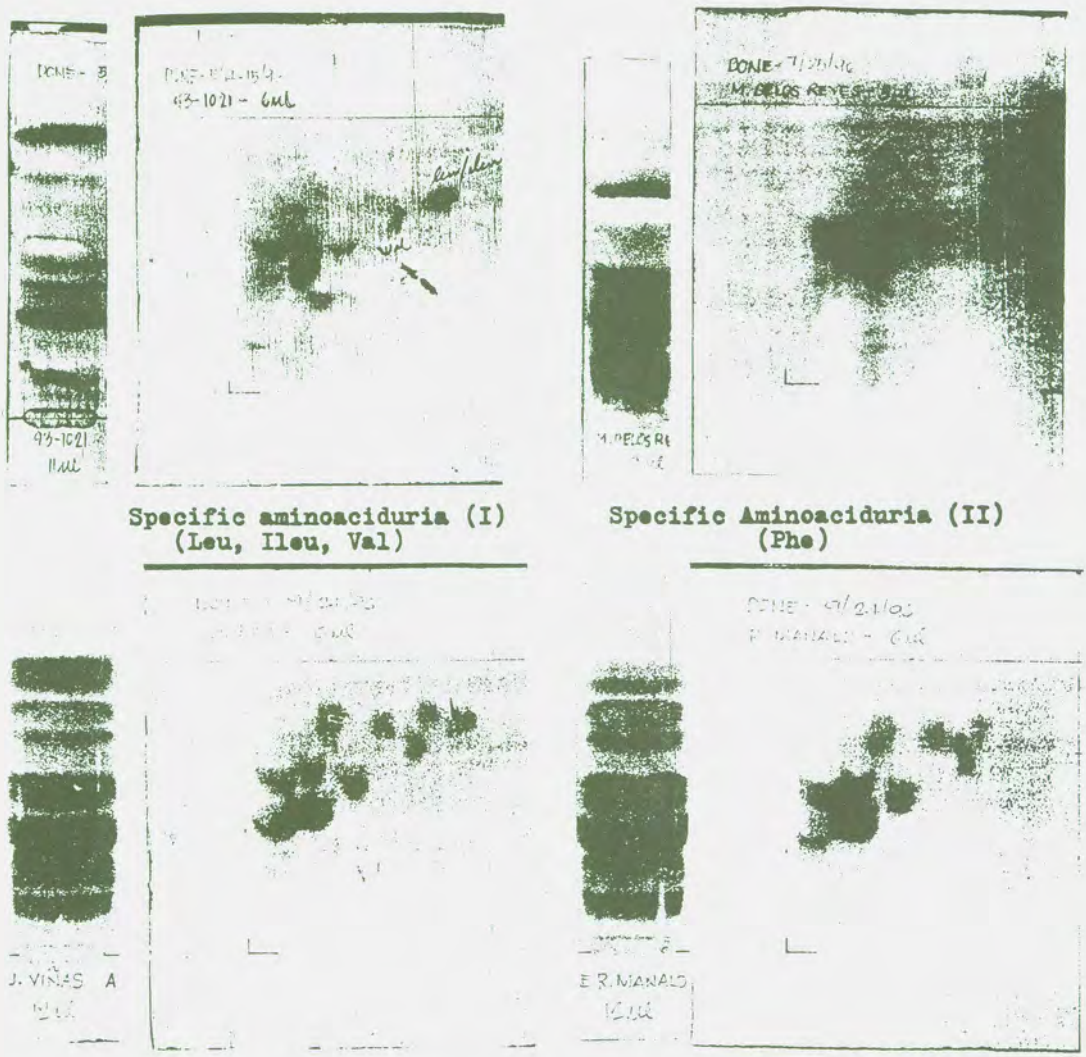
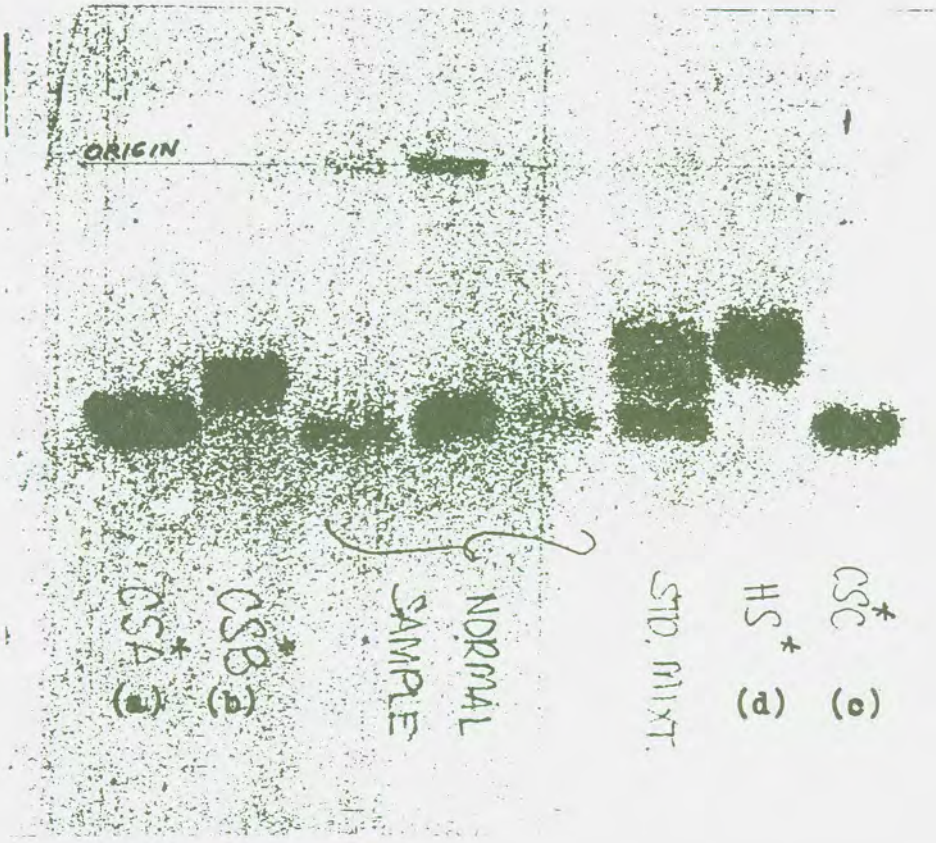


Figure 5. One- and two-dimensional thin layer chromatograms of amino acids of selected urine samples from the case group showing specific aminoaciduria and generalized amino aciduria.



(a) chondroitin sulfate A
(b) chondroitin sulfate B (Dermatan sulfate)
(c) chondroitin sulfate C
(d) heparan sulfate

Figure 6. Electrophoretic patterns of standard preparations of mucopolysaccharides in a mixture and in separate solutions compared to three (3) normal urine samples.

APPENDIX A

INFORMATION SHEET

Pangalan _____ Informant _____

Kapanganakan _____ Kasarian ( ) Babae ( ) Lalake
(Month/Day/Yr.)

Address _____

Case# _____ Clinic/Hospital _____

I.

1. Nagkaroon ba ng sakit ang bata noong nakaraang linggo ( ) Oo ( ) Hindi

   Kung Oo, ano ang mga naging sakit niya? _____

   _____

2. Nagkaroon ba ng matagalang sakit ang bata na ginagamot hanggang sa kasalukuyan? ( ) Oo ( ) Hindi
   Kung mayroon, ano ito?

   ( ) seizures ( ) Failure to thrive
   ( ) Renal/liver condition

3. Alin sa mga gamot na nakalista ang ininom ng bata ng nagkaraang 72 oras?
   Ilista sa ibaba:

   ( ) Phenobarbital ( ) Ampicillin
   ( ) Dilantin ( ) Gentamicin
   ( ) Chloral hydrate ( ) Chloromphenicol
   ( ) Valproic acid ( ) Others: _____

II.

4. Anu-ano ang mga naging sakit ng bata mula nang isilang?
   Ilista sa ibaba at isulat kung anong gulang siya nagkaroon ng gayong sakit.

Sakit	Gulang (buwan/taon)
1.	
2.	
3.	

5. Naospital na ba ang bata mula nang isilang? ( ) Oo ( ) Hindi
   Kung Oo, ilang taong gulang siya? _____
   Ano ang naging sakit niya? _____

6. Naoperahan na ba ang bata? ( ) Oo ( ) Hindi
Kung Oo, ilang buwan o taong gulang siya? _____
Anong operasyon ang ginawa? _____

7. Nasalinan na ba siya ng dugo? ( ) Oo ( ) Hindi
Kung Oo, ilang buwan/taong gulang? _____
Ano ang dahilan? _____

8. Mayroon bang "allergies" ang bata? ( ) Oo ( ) Hindi
Sa anong mga bagay siya may allergy? _____

9. Lagyan ng tsek ang mga bakunang naibigay na sa iyong anak:

( ) BCG ( ) DPT1 ( ) DPT2 ( ) DPT3 ( ) Hepatitis B
( ) OPV1 ( ) OPV2 ( ) OPV3 ( ) Anti-measles (tigdas)

III.

10. Paano isinilang ang bata?

( ) Normal (dumaaan sa puerta, hindi ginamitan ng 'forceps')
( ) Ginamitan ng forceps
( ) Caesarian section

11. Nang isilang ang bata, siya ay: (lagyan ng tsek)

( ) husto sa buwan (term)
( ) kulang sa buwan (premature)
( ) may kakambal (triplet, etc.) ( ) 1st ( ) 2nd ( ) 3rd

12. Pang-ilang siyang ipinagbuntis? _____

13. Nang siya ay ipinagbuntis, ang ina ba ay: (lagyan ng tsek)

( ) nagkasakit, ano ang naging sakit? _____

( ) uminom ng gamot? Anong gamot? _____
_____

14. Nagkaroon ba ng komplikasyon ang panganganak (maselang pag-durugo, problema sa inunan, matagal na labor) ( ) Oo ( ) Hindi

Kung Oo, ano ang naging komplikasyon? _____
_____

15. Nagkaroon ba ng komplikasyon sa pagsilang o sakit ang bata nang isilang? ( ) Oo ( ) Hindi

Ano ito? _____

16. Kinailangan niya bang manatili sa ospital dahil sa kumplikasyon sa pagsilang? pagkakaroon ng sakit? ( ) Oo ( ) Hindi

IV. Impormasyon tungkol sa ina:

17. Isulat ang karampatang sagot

a. Ilang beses nagbuntis _____
b. Ilang beses nakunan _____
c. Ilang beses nanganak nang maaga sa buwan (premature) _____
d. Ilang anak ang nabubuhay sa kasalukuyan?
OB SCORE (- - -)
e. Nagkaroon ba ng anak na patay na nang isilang? Ilan? _____
f. Ilan ang namatay matapos isilang nang buhay? _____
Ilang araw/buwan/taong gulang? _____
Anong sanhi ng pagkamatay? _____
( ) Hindi alam

V. Family History

18. Anu-ano ang mga sakit sa pamilya? (magulang, kapatid ng magulang at kapatid ng ina at ama ng bata/sanggol)

( ) Epilepsy
( ) Failure to thrive
( ) Genetic Disorders

19. Mayroon bang may kapansanan sa iba pang mga anak? ( ) Oo ( ) Hindi
Kung Oo, ( ) Lalake ( ) Babae
Ano ang kapansanan? _____
Pang-ilang anak? _____

20. Mayroon bang may kapansanan sa pamilya? ( ) Oo ( ) Hindi
Kung Oo, alin dito?

Relasyon sa sanggol/bata

( ) Epilipesy _____
( ) Failure to thrive _____
( ) Genetic Disorders _____

21. Mayroon ba sa iyong pamilya o pamilya ng iyong asawa na patay nang isilang o namatay nang kabataan? ( ) Oo ( ) Wala

Kung Oo, ano ang relasyon sa sanggol/bata at ano ang sanhi ng pagkamatay? _____

VI.
Lagyang ng tsek ang mga bagay na nagawa na o kaya nang gawin ng bata mula nang siya's isilang hanggang sa kasalukuyan. Isulat sa kanan kung ilang buwan/taong gulang niya unang nagawa o nakayanang gawin ito.

GAWAIN	ILANG BUWAN/TAONG GULANG UNANG NAGAWA (bilugan kung buwan o taon)
( ) pag-ngiti	_____ buwan/taon
( ) pag-angat ng ulo ng 45° sa nakadapang posisyon	_____ buwan/taon
( ) pag-angat ng ulo ng 90° sa nakadapang posisyon	_____ buwan/taon
( ) hindi na kailangang suportahan ang ulo kapag kinakarga/kaya nang dalhin ang ulo (full head control)	_____ buwan/taon
( ) pagtayo nang may suporta	_____ buwan/taon
( ) pagtayo nang walang suporta	_____ buwan/taon
( ) paglakad nang may suporta	_____ buwan/taon
( ) paglakad nang walang suporta	_____ buwan/taon
( ) pagtakbo	_____ buwan/taon
( ) pagsalita ng "mama" o "dada"	_____ buwan/taon
( ) pagsunod sa utos o pakiusap	_____ buwan/taon
( ) pagbigkas at pag-unawa ng 3 salita	_____ buwan/taon
( ) pagbigkas at pag-unawa ng 10 salita (maliban sa mama at dada)	_____ buwan/taon

VII.

1. Anong uri ng gatas ang ibinigay o pinainom nang sanggol pa? Isulat kung ilang buwan o taong gulang sinimulan at tinigil.

	mula (buwan/taon)	hanggang (buwan/taon)
( ) gatas ng ina	_____	_____
( ) gatas ng baka/kalabaw/kambing	_____	_____
( ) gatas na powdered brand _____ timpla _____	_____	_____
( ) de lata (evap/kondensada) _____ timpla _____	_____	_____

2. Kailan binigyan ng solid food ang bata? _____
   Ano ang binigay? _____

3. Ano ang pinapakain sa bata ngayon? _____

   Gaano kadalas kumain _____
   Gaano karami? _____

4. Ano ang huling kinain ng bata/sanggol? _____

   Kailan pa kinain? _____
   Gaano karami? _____

APPENDIX B

Results of amino acid metabolite screening tests on urine samples of patients referred to the Department of Biochemistry and Molecular Biology, 1993-1996.

	FERRIC CHLORIDE (Phenylketones)	DNPH (α-keto acids)	NITROSO-NAPTHOL (Tyrosine)	NITRO-PRUSSIDE (Cystine)
Positive Results	*greenish, greyish to green gun metal*	*yellow opalescent/ turbid orange/ ppt within 5 mins*	*orange-red color within 2-5 minutes*	*deep purple which fades gradually*
DEVELOPMENTAL DELAY (116)	(-) *clear light yellow to turbid orange*	(-) *clear light yellow to slightly turbid orange*	(-) *clear to turbid light yellow*	(-) *clear slightly pink to clear yellow*
SEIZURE DISORDERS (91)	(-) *clear to turbid greenish yellow*	(-) *clear yellow to turbid orange* (traces) 1	(-) *clear to slightly turbid yellow*	(-) *clear light pink to clear yellow* (+)1 *clear very light cherry red*
MENTAL RETARDATION (16)	(-) *clear light yellow to turbid yellow* (+)9 *gun metal green*	(-) *clear lemon yellow* (+)9	(-) *slightly turbid to turbid yellow*	(-) *clear yellow to clear peach*
METABOLIC DISORDER (8)	(-) *clear light yellow to turbid dark brown*	(-) *clear yellow to turbid orange*	(-) *clear light to turbid yellow*	(-) *clear slightly pink to clear yellow*
AUTISTIC BEHAVIOR (22)	(-) *clear light to turbid yellow* (+)1 *turbid green*	(-) *clear yellow to clear orange* (+)2 *turbide yellow orange*	(-) *clear yellow to slightly turbid yellow orange*	(-) *clear peach to turbid light pink*
CONGENITAL BRAIN ANOMALY (2)	(-) *clear to turbid yellow*	(-) *clear lemon yellow to clear orange*	(-) *clear yellow to slightly turbid yellow*	(-) *clear slightly pink to clear peach*

MULTIPLE SKELETAL DEFORMITIES	(2)	(-) *turbid yellow*	(-) *clear yellow to clear orange*	(-) *slightly yellow*	(-) *turbid yellow*
RICKETS	(2)	(-) *clear light green to turbid yellow*	(-) *clear yellow clear orange*	(-) *clear orange to slightly turbid yellow*	(-) *clear peach to clear yellow*
SEPSIS	(25)	(-) *clear light yellow to clear light green* (+)7 *gun metal green*	(-) *clear lemon yellow* (+)9 *turbid yellow*	(-) *slightly turbid yellow*	(-) *clear pink to turbid light pink*
MENINGITIS	(6)	(-) *clear orange brown to turbid yellow*	(-) *clear yellow* (trace)1 *turbid orange*	(-) *clear light to slightly turbid yellow*	(-) *clear pink to clear yellow*
PNEUMONIA	(9)	(-) *clear greenish yellow to turbid brown*	(-) *clear lemon yellow to slightly turbid orange*	(-) *clear light yellow to slightly turbid light yellow*	(-) *clear pink to clear light yellow*
CYANOSIS	(1)	(-) *turbid orange brown*	(-) *clear yellow orange*	(-) *slightly turbid light yellow*	(-) *clear peach*
HYPOTONIA	(2)	(-) *clear brown*	(-) *clear orange*	(-) *clear yellow*	(-) *clear yellow*
MULTIPLE CONGENITAL ANOMALY	(7)	(-) *clear light yellow to turbid orange*	(-) *clear light yellow to clear golden yellow*	(-) *clear light yellow to slightly turbid light yellow*	(-) *clear pink to clear yellow*
CONGENITAL NEPHROTIC SYNDROME	(2)	(-) *clear brown*	(-) *clear yellow*	(-) *clear yellow*	(-) *clear yellow*
Requests w/o clinical description	(114)	(-) *clear light yellow to turbid yellow* (+)3 *gun metal*	(-) *clear yellow to slightly turbid yellow orange* (trace)2 (+)2 *turbid yellow orange*	(-) *clear golden yellow to slightly turbid yellow*	(-) *clear slightly pink to clear yellow*