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Screening tests for inborn errors of metabolism associated with mental retardation

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Screening Tests For Inborn Errors Of Metabolism Associated With Mental Retardation

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Inborn errors of metabolism and screening tests for their detection have been well publicized in both medical and popular magazines since the dramatic discovery of PKU testing and the ability to prevent mental retardation with low phenylalanine diets. So far, over 50 inborn errors of metabolism have been discovered and new cases are being detected all over the world.

It is now becoming feasible to screen patients for most of the now-known inborn errors of metabolism. It is the purpose of this paper to describe the fewest tests which would cover the greatest number and/or the most important errors of metabolism. This paper will cover those inborn errors which are associated with mental retardation.

Such screening tests would probably not be run on all newborns as the PKU test is now being run. Dr. Richard Stambough and Dr. D. Davidson doubt that amino acid screening tests would be of value in the general population(1). In screening 500 newborns, they turned up only known errors of metabolism which they believed were more easily recognized by simple laboratory, physical and neurological exams. They found a number with high B aminoisobutyric acid excretion levels which had no apparent clinical significance.

At the present time it is suggested that screening tests should be run on: quote,

"

1. Patients with gross malformations.
2. A mentally retarded child with seizures or early death from unknown causes or any previously undescribed syndrome occuring in a sibling or close relative.
3. Any unusual odor of the breath, skin or urine.
4. A retarded child with failure to thrive in the absence of

improper feeding, intestinal or renal malformation.

5. Any child with progressive neurological disorder."(2)

Even though the metabolic error is detected there may be no cure for the child. There are a few in which early detection and therapy may spare a child from severe brain damage and permanent physical disability. There are others in which new means of therapy are being investigated. As we find more and more of these children we are learning about their exact metabolic defect and rational ways to handle them.

AMINOACIDURIA and/or AMINOACIDEMIA

Maple-Syrup Urine Disease

Clinical findings: Onset- 3-5th day of life. Severe mental retardation. Maple syrup odor to urine±. Death may occur during 1st week and usually occurs in the 1st year.

Urine: Aminoaciduria. Valine, leucine and isoleucine increased greatly. Threonine, serine and alanine exceptionally low.

Serum: Valine, leucine and isoleucine increased greatly. Hypoglycemia. (3)

Metabolic defect: A block in oxidative decarboxylation (4).

Treatment: Casein hydralysate diet- low in leucine, isoleucine and valine (5). Low phenylalanine diet (6).

At birth, analysis of blood or urine is not likely to yield significant abnormalities. Paper chromatography of plasma on the 4th day and blood on the 5th day have been adequate. (6)

Phenylketonuria

Clinical findings: Mentally defective, usually severe. Musty odor to urine±, eczema±, blond±, blue eyes± (3).

Urine: Aminoaciduria. Increased phenylalanine.

Blood: Increased phenylalanine in serum.

Metabolic defect: Inactivity of the enzyme phenylalanine hydroxylase (6).

Treatment: Low phenylalanine diet.

Analysis of blood for increased concentration of phenylalanine must be taken no sooner than 24 hours after the beginning of milk feedings. A second blood test should be taken at 4-6 weeks (7).

Particular care must be exercised in interpreting results of tests in low birth - weight infants for accumulation of phenylalanine and the urinary excretion of reducing substances because these children may give false positives.(7)

Hypervalinemia

Clinical findings: Failure to thrive, vomiting, nystagmus, and hyperkinesia.

Urine: Valine levels increased. Leucine and isoleucine levels normal.

Blood: Valine increased. Leucine and isoleucine levels normal.

Metabolic defect: Unknown.

Treatment: Possibly diet low in valine, leucine and isoleucine.(8)

Hypermethioninemia

Clinical findings: Irritability and progressive drowsiness. An unusual odor developing between 2-8 weeks. Death at 11-12 weeks with severe bleeding tendencies.

Urine: Generalized aminoaciduria. Methionine increased greatly.

Blood: Increase in methionine.

Metabolic defect: Not known.

Treatment: Not known. (8)

Methionine Malabsorption Syndrome

Clinical findings: Mental deficiency, convulsions, attacks of hyperpnea and periodic diarrhea. White hair, blue eyes+.(9)

Urine: Large amounts of alpha-hydroxybutyric acid.

Feces: Large amounts of methionine and branched chain amino acids.

Blood: Non contributory.

Metabolic defect: Not known.

Treatment: Low methionine diet (8).

Tryptophanuria

Clinical findings: Dwarfism, Mental retardation, photosensitivity, gait disturbances and pellagra-like rash.

Urine: Tryptophan levels increased. No increase in indican or indol acetic acid.

Blood: Slight increase in tryptophan.

Metabolic defect: Tryptophan pyrrolase and/or formamidase.

Treatment: Not known. Nicotinic acid could be tried. (8)

Tyrosinemia

Clinical Features: Multiple tubular reabsorption defects, hepatosplenomegaly, nodular cirrhosis of the liver, Vit. D-resistant rickets and slight mental retardation in some patients.

Urine: Increased tyrosine.

Blood: Increased tyrosine. (6)

Metabolic defect: Lack of p-hydroxyphenylpyruvic acid.

Treatment: Diet low in phenylalanine and tyrosine. Some infants have been reported who have had a transient tyrosinemia. These tyrosine levels usually drop rapidly and this does not appear to have any clinical significance. These children do not need a special diet and may even be harmed by a too low phenylalanine diet. (8)

Prolinemia Type I

Clinical findings: Congenital anomalies of the kidney(6). Nerve deafness and mantal retardation.

Urine: Proline levels increased. Hydroxyproline increased. Glycine increased.

Blood: Proline increased.

Metabolic defect: Proline oxidase. (8)

Prolinemia Type II

Clinical findings: Possible mild mental retardation and convulsions.

Urine: Proline increased.

Blood: Proline increased.

Metabolic defect: delta-pyrolline-5-carboxylate dehydroginase.(8)

Hydroxyprolinemia

Clinical findings: Mild to severe mental retardation. Possibly microscopic hematuria(10).

Urine: Increased hydroxyproline.

Blood: Increased hydroxyproline.

Metabolic defect: Hydroxyproline oxidase.

Treatment: None known. (6)

Hyperglycinemia

Clinical findings: Episodes of metabolic acidosis and ketonuria with vomiting, lethargy or coma. Hypogammaglobulinemia and neutropenia, periodic purpura and thrombocytopenia. Developmental retardation. Onset-neo-natal period. Osteoporosis (11).

Urine: Increased levels of glycine.

Blood: Marked increase of glycine, serine, alanine, isoleucine and valine (12).

Treatment: Drastic reduction of protein. Breast fed infants may be protected until fed a supplementary diet (13).

Glycinemia with hypo-oxaluria

Clinical findings: Severe mental retardation, convulsions, spasticity, episodes of excessive sweating.

Urine: Glycine increased. Oxylate decreased.

Blood: Glycine increased.

Metabolic defect: Not known.

Treatment: Possibly a low protein diet. (8)

Hyperammonemia

Clinical findings: Mental retardation, episodes of vomiting, failure to thrive.

Urine: Glutamine increased.

Blood: Increased ammonia levels.

CSF: Glutamine increased.

Metabolic defect: Disorder of the ornithine cycle of urea synthesis.

Treatment: Drastic reduction of protein intake. (14)

Citrullinemia

Clinical findings: Seizures, developmental regression, vomiting, and alkalosis (15).

Urine: Increased citrulline.

Blood: Increased citrulline. Post prandial hyperammonemia.

CSF: Increased amounts of citrulline.

Metabolic defect: Possibly Argininosuccinic acid synthase (8).

Treatment: Thyroid hormone. Decreased protein intake may help (6).

Argininosuccinic Aciduria

Clinical findings: Mild to moderate mental retardation. Friable and tufted hair+.

Urine: Increased amounts of argininosuccinic acid.

Blood: Low to normal concentrations of argininosuccinic acid. Post prandial elevation of blood ammonia.

CSF: Increased amounts of argininosuccinic acid. (16) (17)

Treatment: Possibly a low protein diet in frequent feedings with adequate arginine intake (6).

Metabolic defect: Probably subnormal activity of ASA ase in the RBC's (6).

Hyperlysinemia

Clinical findings: Impaired sexual development, lax ligaments, hypotonic muscles, seizures, mental and physical retardation and abnormal EEG.

Urine: Lysine increased.

Blood: Lysine increased. Persistent hyperlysinemia even on a low protein diet. (20)

Metabolic defect: Not Known.

Lysine intolerance

Clinical findings: Vomiting and episodes of coma from birth. Convulsions and spasticity.

Urine: Normal.

Blood: High concentrations of blood ammonia with protein intake. Arginine and lysine increased on high protein diet. Normal on a low protein diet. (21)

Cystathioninuria

Clinical findings: Mild to moderate mental retardation.

Urine: Increased amounts of cystathionine. (22)

Blood: Normal or increased amounts of cystathionine.

Metabolic defect: Not known.

Treatment: Pyridoxine has caused a reduction in cystathionine excretion (23).

Histidinemia

Clinical Findings: Mental retardation± (18).
Most have a defect or retarded development of speech. (6)

Urine: Histidine levels increased. Excessive imidazole-pyruvic, imidazolelactic and imidozolacetic acid (19).

Blood: Histidine levels increased.

Metabolic defect: Absence of histidase.

Treatment: Probably a diet low in histidine (6).

Cystinuria

Clinical Findings: May vary from no clinical features, to renal calculi, to mental retardation with atypical osteogenesis imperfecta (24).

Urine: Increased levels of Cystine. May also have increase in lysine, arginine and ornithine.

Blood: Normal.

Homocystinuria

Clinical Findings: Mental retardation±, fine fair hair, dislocated optical lenses, malar flush. Skeletal abnormalities have included genu valgum, pes cavus, pectus excavatum

and kyphoscoleosis and arachnodactyly. Medial degeneration of the aorta or elastic arteries resembling Marfans. Hypercoaguability of blood and thrombo embolic disease. (25) (27)

Urine: Increased amounts of homocystine.

Blood: Increased amounts of homocystin.

Metabolic defect: Cystathionine synthase(8).

Treatment: Possibly a diet low in methionine with cystine supplement (26).

Sarcosinemia

Clinical findings: Mental retardation, difficulty swallowing, failure to thrive (28).

Urine: Increased levels of sarcosine.

Blood: Increased levels of sarcosine.

Metabolic defect: Sarcosine dehydrogenase (8)?

GENERALIZED AMINO ACIDUREAS AND AMINO ACIDEMIAS

Galactosemia

Clinical findings: Vomiting, lethargic, failure to thrive, hepatomegaly, jaundice and cataracts occur in the neonatal period.

Urine: Galactose present. Generalized aminoaciduria, and generalized proteinuria.

Blood: Blood sugar increased.

Metabolic defect: Decrease or absence of P-gal-uridyl-transferase.(3)

Treatment: Withholding all milk and milk products.

Urine tests will not be positive unless the child has been on milk

feedings. In addition to cases of classic galactosemia there are patients who may not be detected for weeks or months. Frequently these patients have had a "milk intolerance" in their early days of life and have been placed on milk substitutes (6).

Hartnup's Disease

Clinical manifestations: Mental defect mild, photosensitivity, pellagra-like rash and cerebral ataxia (8).

Urine: Generalized aminoaciduria. Tryptophane increased. Indol excretion markedly increased.

Blood: Tryptophane probably decreased.

Feces: Increase in tryptophane.

Metabolic defect: Transport of lysine and ornithine by jejunal cells is grossly impaired (29).

Treatment: Nicotinamide or combined Vit. B preparations have value in acute exacerbations but do not influence the excretion of amino acids, cerebellar ataxia or mental deterioration (30).

Lowe's Syndrome

Clinical findings: Males have been the only reported cases. Mental retardation, glaucoma, cataracts, hypotonic with choreo-athetoid movements. Virtually all have been blind. Some cases have had some light perception.

Urine: Generalized aminoaciduria.

Blood: Metabolic acidosis. (31)

Metabolic defect: Not known.

Treatment: Not known.

ABNORMAL SUGAR METABOLISM

Galactosemia (see page 10.)

Urine: Galactose present. Generalized aminoaciduria. Generalized proteinuria.

Blood: Blood sugar increased.

Fructosemia (Fructose intolerance)

Clinical findings: Failure to thrive, vomiting, jaundice and hepatosplenomegaly+.

Urine: Fructose is increased. Albuminuria.

Blood: Fructose is increased. Hypoglycemia.

Metabolic defect: Defect in hepatic enzyme fructose-1-PO_4 aldolase.

Treatment: No fruits or sucrose or fructose containing food. (6)

Idiopathic Hypoglycemia

Clinical findings: Weakness, flushing, sweating, speech disturbances and visual disturbances. (3)
May have staring gaze, coarse body and extremity twitching and convulsions. If not recognized early, this may cause permanent brain damage (32).

Urine: Negative.

Blood: Oral glucose tolerance test or 2 hour post-prandial give low sugar levels.

Treatment: ACTH, 5-10 mg. every 2-3 days (3).

Hypoglycemia due to leucine intolerance.

Clinical findings: Those of hypoglycemia.

Urine: Negative.

Blood: Low blood sugars with a decline of blood sugar levels

greater than 50% 20-40 min. after administration of leucine (33).

Treatment: Leucine free diet.

DEFECT OF PURINE METABOLISM

Hyperuricemia -Juvenile Gout

Clinical findings: Mental retardation, choreo-athetosis, self-destructive biting. So far it has only been found in males. Death usually occurs in childhood (34).

Urine: Uric acid is increased. Uric acid stones may be formed.

Blood: Serum uric acid increased.

Metabolic defect: Not known. Formation of uric acid from glycine exceeded that of the controls by 200x. (35) (36)

Treatment: Possibly probenecid or xanthine oxidase inhibition (8).

DEFECTS OF CALCIUM METABOLISM

Idiopathic Hypercalcemia of Infancy

Clinical findings: Failure to thrive with episodes of vomiting and constipation (37). Mental retardation, physical and motor underdevelopment, characteristic facies, usually with congenital heart disease (38). There are mild to severe forms.

Urine: Often impaired renal function.

Blood: Increased serum calcium.

Treatment: Calcium free diet. Restriction of Vit. D and possible administration of cortisone (39). Treatment with Sodium sulfate could be tried (40).

Blue Diaper Syndrome

Clinical features: Blue color of diaper often appearing after diaper has been placed in a diaper pail, irritability, failure to thrive, infections, constipation and recurrent unexplained fever. (41) (8)

Urine: Indican and other indoles increased.

Blood: Increased levels of calcium.

Metabolic defect: In the intestinal transport of tryptophane.

Treatment: Decrease the intake of calcium. Stop Vit. D (41).

Pseudo Hypoparathyroidism

Clinical findings: Some degree of mental defeciency, short stature, round facies, stubby hands and convulsions.

Urine: Negative.

Blood: Decreased levels of calcium. Increased levels of inorganic phosphate. No response to parathormone.

Metabolic defect: This appears to be due to a lack of end organ response.

Treatment: A diet high in calcium plus Vit. D_2. Benemid may also be used. (42)

Craig, et al reported the cases of 26 infants who developed hypocalcemic tetany within 40 min. to 36 hours after birth. Most of these children recovered spontaniously and the others recovered with calcium administration. These children should not be mistaken for pseudo hypoparathyroid children (43).

DEFECTS OF THYROID METABOLISM

Hypothyroidism

Clinical findings: Large tongue, lethargic, poor appetite, subnormal temperature. An umbilical hernia may be present. Progressive physical and mental retardation.

Urine: Negative.

Blood: PBI decreased. I_{131} decreased. (44)

Metabolic defect:

Type I Sporadic Cretinism

This is usually caused by a complete absence of thyroid gland or only a rudiment.

Type II Sporadic Goitrous Cretinism

In these cases there is an enlarged thyroid. The metabolic defect is genetically determined.

The defects are the following:

1. Iodide-trapping defect.
2. Iodide organification defect (most freguent (45).
3. Coupling defect
4. Deiodinase defect
5. Abnormal serum iodoprotein.
6. Pendred Syndrome. The same metabolic defect as 2. but has a familial eighth nerve deafness(6).

Treatment: Thyroid hormone. (44)

DEFECTS OF LIPID METABOLISM

Gaucher's Disease

Clinical features: I Chronic "adult" type. This is the most common form. Splenomegaly, anemia and osteoporosis.

II "Infantile" type. Progressive physical and developmental retardation. Hepatosplenomegaly, cachexia and death usually before the first year (3).

Urine: Negative.

Blood: Acid phosphatase is greatly increased (6).

Bone Marrow: Pale, poorly staining material in large multinucleated cells. ("Gaucher cells").

Metabolic defect: Not known.

Treatment: Splenectomy is helpful but is not a cure (6).

Tay-Sachs Disease

Clinical findings: Progressive retardation in development, paralysis, dementia and blindness, associated with a cherry-red spot in the retina. It begins to become clinically evident by 4 to 6 months of age. It is invariably fatal.

The characteristic pathological alterations are restricted to the nervous system.

Metabolic defect: Not known. There is a progressively increasing accumulation of a specific monosialoganglioside in the cytoplasm of ganglion cells.

Treatment: None known. (6)

Metachromatic Leukodystrophy

Clinical findings: The child appears normal in the first year or two. Progressive muscular weakness and incoordination develop. Eventually there is a complete decerebrate rigidity, and death usually occurs between the third and sixth year.

Urine: Lipid-like granules found in stained sediment.

Metabolic defect: Not known but there is a marked excess of sulfatide in the white matter.

Treatment: Not known.

DEFECTS OF MUCOPOLYSACCHARIDE METABOLISM

Hurler's Syndrome

Clinical findings: Cloudy corneas, hepatosplenomegaly, mental deficiency, skeletal changes and dwarfism (44).

Urine: Increased amounts of Chondroitin sulfuric acid B and heparin monosulfuric acid (6).

Bone Marrow: Accumulation of mucopolysaccharide granules which can be recognized by conventional staining of bone marrow (46).

Metabolic defect: Possibly a defective binding of chondroitin sulfuric acid to protein.

Treatment: Large doses of adrenocortical hormone have been used with variable results (6).

Morquio's Disease

Clinical findings: Dwarfism and deformities of the spine and chest. Development appears to be normal until the infant

begins to walk. Mental development is not impaired.

Urine: Increased excretion of mucopolysaccharides. (44)

Metabolic defect: Not known.

Treatment: Not known.

Morquio-Ullrich's Disease

Clinical findings: Same as Morquio's disease but also mental retardation, corneal cloudiness,(44) hepatosplenomegaly and deafness(6).

Urine: Increased excretion of mucopolysaccharides.

Metabolic defect: Not known.

Treatment: Not known.

SCREENING TESTS

Screening test for Amino Acids

I Paper Chromatography Method

This is a good screening method as it uses only a drop of urine and a drop of blood. Fifteen samples can be run on a single chromatogram and five chromatograms could be run simultaneously (48).

Certain precautions can be taken to insure reproducibility of results.

1. All blood and urine are collected before breakfast.
2. The child should have been on a protein diet for at least 24 hours.
3. A plasma sample of a known amino acid composition should be run on each square.
4. Specific gravity should be run on each urine as too dilute urine might cause an amino-aciduria to be overlooked; or conversely very concentrated urine might lead to a wrong interpretation of a normal pattern.

Rosanova (47) also recommended:

5. 200 cc of solvent placed in the tank 1 hour before use.
6. A constant room temperature.
7. A room free of all fumes and chemical odors.
8. Spraying is to be done under a ventilated hood in a different room.
9. The tank cover is applied immediately after loading the tank with solvent or papers.
10. The solvent must always be just below the spot and never above it.

Both blood and urine should be run on each patient as some amino-acidopathies are increased in one but not the other.

Method (48)

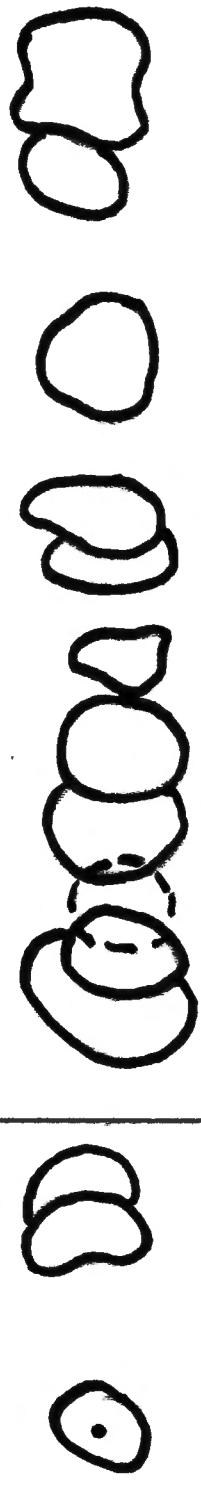
Whole blood from heels of infants are drawn into heparinised capillary tubes. These are spun down and the plasma is transferred into a 10 microliter delivery pipette and applied to Whatman 3 MM filter paper, 10 in. square (with prepunched corner holes). The samples were spaced no less than 1.5 cm. apart on a line drawn 4 cm. from the bottom edge. A drop of urine is applied in the same manner.

The chromatograms are developed overnight in freshly mixed n-butanol, acetic acid, and water (12/3/5). After drying for one hour in a stream of air, the chromatograms are stained with a ninhydrin-isatin mixture (ninhydrin 0.25% w/v, isatin 0.01% w/v, and lutidine 1% v/v in acetone), and heated at 75° C for 15 min. They should be inspected by transmitted light after heating and again in 24 hours.

For further identification of hydroxyproline and citrulline, the stained chromatogram is cut at line A (see figure 1) and the upper portion is overstained with Ehrlich's reagent. The two compounds will give a purple and orange-brown color respectively while other aminoacids will loose their ninhydrin color without reacting with the new stain. The other portion of the ninhydrin chromatogram is stained with Pauley's reagent; histidine gives a red-brown color reaction.

Results: Complete separation of every amino acid is not obtained. Phenylalanine can not be detected consistently when its concentrations are below 8 mgm. per 100 ml. At the low range its initial grey-brown color enhances its detection but for this advantage the chromatograms must be inspected just after heating.

A few newborns show a moderate transient tyrosinemia in the first week



-LEUCINE

-ISOLEUCINE

-PHENYLALANINE

-VALINE

-METHIONINE

TRYPTOPHAN

-TYROSINE

- alpha-AMINO-N-BUTYRIC ACID

-PROLINE

-ALANINE

-THREONINE

-GLUTAMIC ACID

-HYDROXYPROLINE

-GLYCINE, SERINE

-GLUTAMINE, CITRULLINE

-ARGININOSUCCINIC ACID

A

-HISTIDINE

-LYSINE, ARGININE, ORNITHINE

-CYSTATHIONINE

-ORIGIN (PROTEIN SPOT)

Figure 1 (48)

Ascending chromatography

	Color Reactions	
Aminoacid	Ninhydrin-isatin	Other reagents
Isoleucine	Purple	....
Leucine	Purple	....
Phenylalanine	Grey-brown	....
Valine	Purple	....
Methionine	Purple	....
Tryptophan	Purple	....
Tyrosine	Brown-grey	....
a-amino-n-butyric acid	Blue-purple	....
Proline	Yellow--darkens with standing	....
Alanine	Purple	....
Threonine	Purple	....
Glutamic acid	Purple	....
Hydroxyproline	Magenta	Red-purple(Ehrlich's)
Glycine	Brown-orange	....
Serine	Purple-grey	....
Glutamine	Purple	Brown-orange(Ehrlich)
Citrulline	Purple	"
Aspartic acid	Blue	"
Argininosuccinic acid	Grey-purple	....
Histidine	Grey	Brown-red(Pauly)
Arginine	Purple	....
Lysine	Purple	....
Ornithine	Purple	....
Cystathionine	Grey-blue	....

of life. Other amino acids have been reported to be transiently excreted in excess amounts so repeat chromatographs and further studies should be done on all positive cases.

Lead poisoning may also give rise to increased amino acid excretion (49).

Homocystine and Cystine do not appear on this method of chomatography. As a screening proceedure for these amino acids the cyanide-nitroprusside test may be used (50).

II Cyanide-nitroprusside test

Method (3)

Reagents: 5% sodium cyanide solution, freshly prepared. CAUTION!
5% sodium nitroprusside, freshly prepared.

Procedure: To 5 ml. of urine, 2 ml. of a 5% sodium cyanide solution is added and the reaction is allowed to proceed for 10 min. A few drops (5 usually) of 5% nitroprusside solution are added and thoroughly mixed. Normal urine shows a pale brown or occasionally a very faint flesh color while urine containing cystine shows a rather stable magenta color.

Sarcosine is a known amino acid intermediate in the one-carbon cycle, and is between dimethylglycine and glycine (8). It is not known if this could be differentiated by either of these two tests.

Screening tests for abnormal Sugar metabolism

I Benedict's Test (49)

Method: Five drops of urine and 10 drops of distilled water are placed in a clean test tube. A copper-reduction tablet (Clinitest) is added. The reaction is read when the boiling ceases, at fifteen to thirty seconds.

Results: A positive test is a green to brick red color, depending upon the amount of reducing substances in the urine. The advantage of this test is that any reducing substance, not only glucose, will give a positive reaction. The usual "dip-stick" method of testing urine is specific for glucose. Benedict's test is positive when the urine contains glucose, galactose, fructose, lactose, mannose or other reducing substances (for example, in phenylketonuria, alkaptonuria, tyrosyluria and tyrosinosis the phenolic compounds may give a positive test). Lead poisoning may also give a positive Benedict's test.

It has been recommended by the Committee of Fetus and Newborns that every newborn should have a test for reducing substances in the urine on the day of discharge from the hospital (7).

II Fasting and two hour post-prandial blood sugar

Hypoglycemia and hyperglycemia may be detected by these tests.

Test for hyperuricemia

Serum uric acid.

Test for defect of Calcium metabolism

Serum Calcium.

Test for Thyroid disorders

PBI

Screening tests for Lipid Metabolic defects

I Plasma acid phosphatase--Gaucher's

II Metachromatic Stain of urinary sediment

Method: (49) Twelve to 15 ml. of a very fresh, clean urine is centrifuged, and the supernate discarded. Two drops of stain

(2% methylene blue in water or 2% toluidine blue O in water) are added to the sediment, mixed well and allowed to stand for several minutes. A drop is transferred to a clean cover slip and slide. Again, this is allowed to stand for several minutes and examined under high-dry or oil immersion for lipid-like granules varying in size from approximately that of a red cell up to the size of a megakaryocyte. These granules may also appear within epithelial cells. The granules will appear golden brown if toluidine blue O is used, and a brilliant red with methylene blue.

Results: A positive test is seen typically in metachromatic leukodystrophy but may also be seen in Tay-Sachs disease and other lipid diseases involving the central nervous system.

Screening test for defective Mucopolysaccharide Metabolism.

The Cetyltrimethylammonium bromide test (49)

Method: One ml. of reagent (5% solution of Cetyltrimethylammonium bromide (hexadecyltrimethylammonium bromide) in 1 M citrate buffer, pH 6.0) is added to 5 ml. of clean, fresh urine at room temperature (a cold urine will invariably give a positive test) and mixed well. The mixture is allowed to stand at room temperature and read at 30 min.

Results: This is a test for an increase in urinary mucopolysaccharides. A positive test is one that gives a heavy, flocculent precipitate. This reaction is almost immediate in Hurler's but may be quite delayed and much less obvious in Morquio-Ullrich syndrome. Large numbers of cells will also give a false positive reaction.

Cost: Any group of screening procedures will be difficult to evaluate as to cost per test. For example in paper chromatography analysis,

Once the equipment has been purchased, the chemicals prepared, and a technologist available to perform the tests, 15-75 tests could be run at the same time. Thus it would probably be necessary to run screening tests at a medical center where several could be run at once.

The following lists of prices are only approximate. They are based on tests which are now run in our lab and based on tests which are similar in amount of chemicals used and time needed to run the test.

The amount of blood needed is calculated by assuming that 2 ml. of whole blood will furnish 1 ml. serum or plasma.

TEST	ml. urine	ml. serum	cost
Paper Chromatography	few drops	few drops	$10.00 approx
Cyanide-nitroprusside	5		2.00 approx
Benedict's	5 drops		1.00(51)
Blood sugar @2	4 ml. blood	(whole)	6.50(51)
Uric acid		1	4.00(51)
Serum calcium		2	5.00(51)
PBI		2	5.00(51)
Acid Phosphatase		1	5.00(51)
Metachromatic stain	12-15		2.00 approx
Cetyltrimethylammonium	5		2.00 approx
Totals	25 ml.	16 ml. whole blood	$42.50

SUMMARY

A series of screening procedures is presented for use in the evaluation of the mentally retarded or neurologically abnormal child.

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