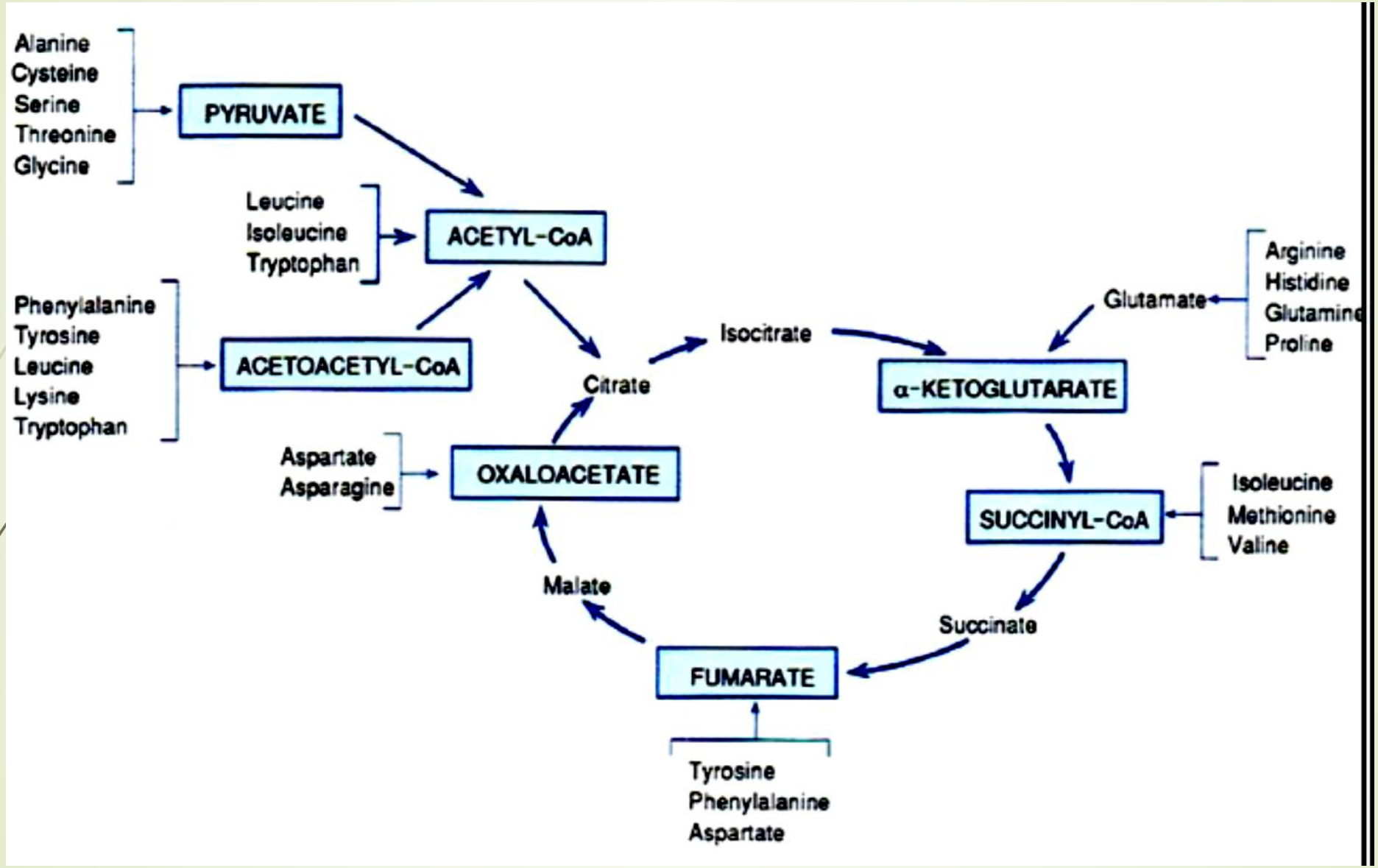
Amino acid metabolism and plasma proteins

Amino acids

- Amino acids in blood are used in:
  - Synthesis of plasma, intracellular and structural proteins
  - Synthesis of nonprotein nitrogen containing compounds: purines, pyrimidines, porphyrins, creatine, histamine, thyroxine, epinephrine and coenzyme NAD
  - Body energy: 12-20% of energy is due to proteins
  - The ammonium produced during deamination of amino acids is converted into urea in liver

TABLE 8-1. AMINO ACIDS REQUIRED IN THE SYNTHESIS OF PROTEINS

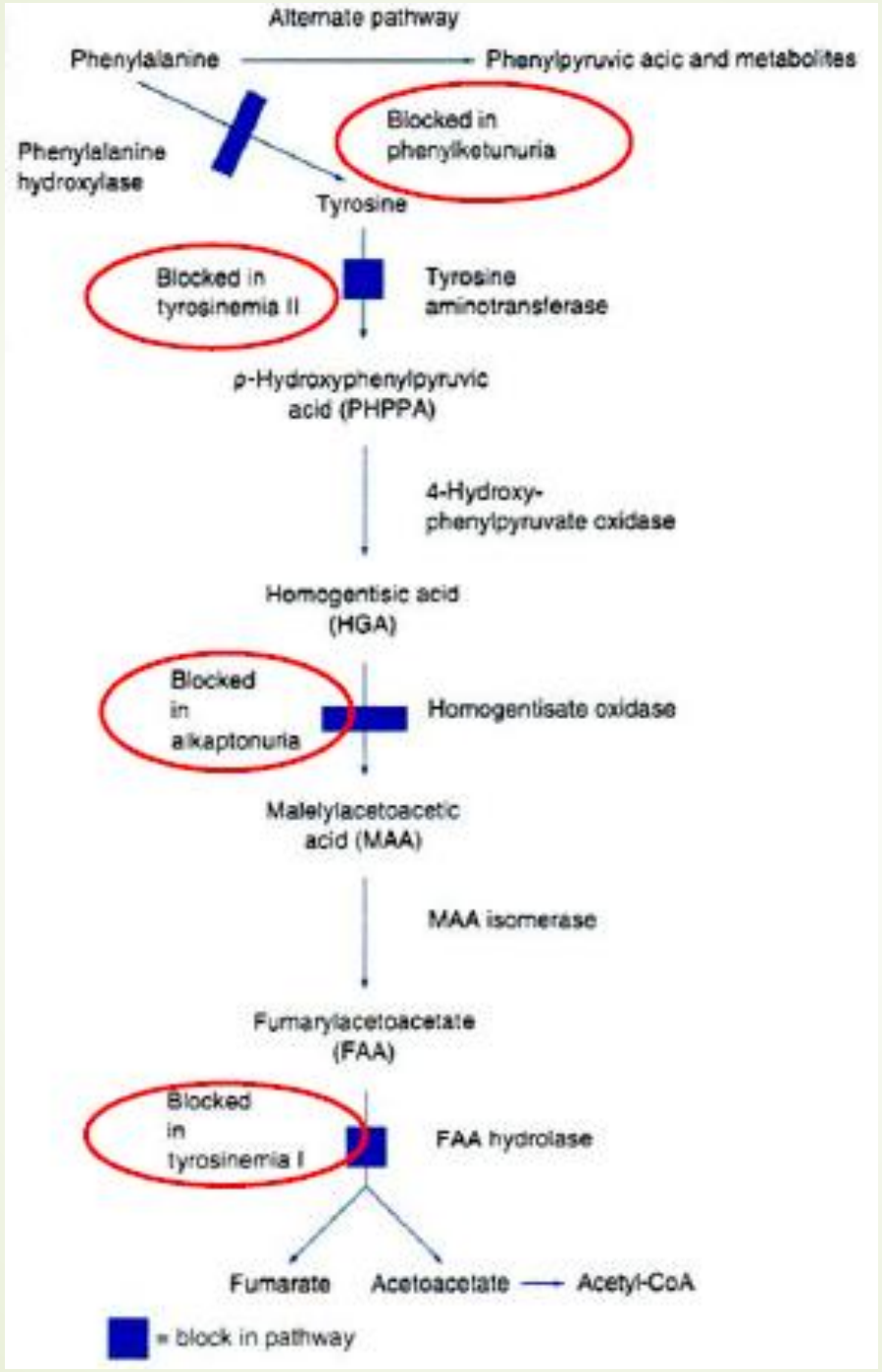
AMINO ACID	R
Glycine (Gly)	—H
Alanine (Ala)	—CH_3
Valine (Val)*	—CH(CH_3)—CH_3
Leucine (Leu)*	—CH_2—CH(CH_3)—CH_3
Isoleucine (Ile)*	—CH(CH_3)—CH_2—CH_3
Cysteine (Cys)	—CH_2—SH
Methionine (Met)*	—CH_2—CH_2—S—CH_3
Tryptophan (Trp)*	—CH_2— (indole)
Phenylalanine (Phe)*	—CH_2— (phenyl)
Asparagine (Asn)	—CH_2—C(=O)—NH_2
Glutamine (Gln)	—CH_2—CH_2—C(=O)—NH_2
Serine (Ser)	—CH_2—OH
Threonine (Thr)*	—CH(OH)—CH_3
Tyrosine (Tyr)	—CH_2— (phenyl)—OH
Lysine (Lys)*	—CH_2—CH_2—CH_2—CH_2—NH_2
Arginine (Arg)	—CH_2—CH_2—CH_2—N(H)—C(=NH_2)—NH_2
Histidine (His)*	—CH_2— (imidazole)
Aspartate (Asp)	—CH_2—COOH
Glutamate (Glu)	—CH_2—CH_2—COOH
Proline (Pro)†	(pyrrolidine)—COOH


Alanine
Cysteine
Serine
Threonine
Glycine
PYRUVATE
Leucine
Isoleucine
Tryptophan
ACETYL-CoA
Phenylalanine
Tyrosine
Leucine
Lysine
Tryptophan
ACETOACETYL-CoA
Citrate
Isocitrate
Glutamate
Arginine
Histidine
Glutamine
Proline
α-KETOGLUTARATE
Aspartate
Asparagine
OXALOACETATE
SUCCINYL-CoA
Isoleucine
Methionine
Valine
Malate
Succinate
FUMARATE
Tyrosine
Phenylalanine
Aspartate

Aminoacidopathies

- Can be in the activity of specific enzyme in the metabolic pathway
- Membrane transport system for amino acids

- Diseases to talk about:
  - Phenylketonurea
  - Maple syrup urine disease (MSUD)
  - Homocystinuria
  - Argeninosuccinic aciduria and citrullinemia
  - Cystinurea


Alternate pathway
Phenylalanine
Phenylpyruvic acic and metabolites
Blocked in phenylketunuria
Phenylalanine hydroxylase
Tyrosine
Blocked in tyrosinemia II
Tyrosine aminotransferase
p-Hydroxyphenylpyruvic acid (PHPPA)
4-Hydroxy-phenylpyruvate oxidase
Homogentisic acid (HGA)
Blocked in alkaptonuria
Homogentisate oxidase
Malelylacetoacetic acid (MAA)
MAA isomerase
Fumarylacetoacetate (FAA)
Blocked in tyrosinemia I
FAA hydrolase
Fumarate
Acetoacetate
Acetyl-CoA
= block in pathway

Phenylketonuria (PKU)

- An autosomal recessive genetic disorder characterized by a deficiency in the hepatic enzyme phenylalanine hydroxylase (PAH)
- The PAH gene is located on chromosome 12
- More than **four hundred** disease-causing **mutations** have been found in the PAH gene
- PAH is necessary to metabolize the amino acid phenylalanine to tyrosine
- When deficient, phenylalanine accumulates to a level > 1200 µmol/L and metabolized by alternative pathways.

Phenylketonuria (PKU)

- The metabolites which are detected in blood and urine include:
- Phenylpyruvic acid (which known as phenylketone): which is the product of deamination of phenylalanine
- phenyllactic acid: which is the reduction product of phenylpyruvic acid
- Phenylacetic acid which is produced by decarboxylation and oxidation of phenylpyruvic acid
- And phenylacetylglutamine: which is the glutamine conjugate of phenylacetic acid

- These metabolites give urine musty odor

Phenylketonuria (PKU)

- Variants of the disease result from partial deficiencies of PAH activity and are typically classified as:
  - Mild PKU if phenylalanine levels are between 600 and 1200 µmol/L
  - Non-PKU mild hyperphenylalaninemia which present with phenylalanine levels in the range of 180-600µmol/L and no accompanying accumulation of phenylketones.
- The normal limits for serum phenylalanine levels for full term, normal weight newborns range from 1.2 to 3.4 mg/dL (70-200 µmol/L)

Phenylketonuria (PKU)

- A rarer of the disease occurs when PAH is normal but there is a defect in the biosynthesis of the **cofactor tetrahydrobiopterin (BH4)** by the patient which is necessary for proper activity of the enzyme (for PA, tyrosine and tryptophan hydroxylation)
- It results in hyperphenylalaninemia, that are not responsive to dietary treatment
- Examination of urinary proteins is helpful in diagnosis
- Although cofactor defects are rare, they must be identified so that appropriate treatment can be initiated
- Patients must be given the active cofactor a long with the neurotransmitter precursor L-dopa and 5-OH tryptophan

Phenylketonuria (PKU)

- Left untreated, this condition can cause problems with brain development, leading to progressive mental retardation and seizures
- In infants and children, the deterioration of brain function begins in the second or third week of life
- Brain damage can be avoided if the disease is detected at birth and the infant is maintained on a diet containing very low level of phenylalanine and high levels of tyrosine
- There is no cure. Damage done is irreversible so early detection is crucial

Screening for PKU

- The Guthrie bacterial inhibition assay:
- Spores of the organism Bacillus subtilis are incorporated into an agar plate that contains β2-thienylalanine, a metabolic antagonist to B. subtilis growth.
- A filter paper disk impregnated with blood from the infant is placed on the agar
- If the blood level exceeds the range of 2-4 mg/dL, the phenylalanine counteracts the antagonists and bacterial growth occurs.
- To avoid false-negative results, the infant must be at least 24 hours old to ensure adequate time for enzyme and amino acid levels to develop
- The sample should be taken before administration of antibiotics or transfusion of blood or blood products
- Premature infant can show false positive results due to the immaturity of the liver enzyme systems

Other screening methods

- Microfluorometric assay: The direct measurement of phenylalanine in dried blood filter disks:
  - This method is quantitative, more adaptable to automation, and is not affected by presence of antibiotics.
- The procedure is based on the fluorescence of complex formed of phenylalanine-ninhydrin-copper in the presence of dipeptide (i.e. L- leucyl-L-alanine).
- The test requires pretreatment of the filter paper specimen with trichloacetic acid (TCA)
- The extract is then reacted with microtiter with a mixture of ninhydrin, succinate, and leucylalanine in the presence of copper tartarate.
- The fluorescence of the complex is measured using excitation/ emission wavelengths of 360 nm and 530 nm, respectively
- For quantitative methods, HPLC or tandem mass spectrometry (MS/MS) are used

Other screening methods

- Urine testing for phenylpyruvate can be used for diagnosis in questionable cases and for monitoring of dietary therapy
- The test which may be performed by tube or reagent strip test involves the reaction of ferric chloride with phenypyruvic acid in urine to produce a green color
- Prenatal diagnosis and detection of carrier status in families with PKU is now available using DNA analysis
- Analysis using cloned human PAH cDNA, has revealed the presence or numerous restriction fragment length polymorphism in the PAH gene

Tyrosinemia and related disorders

- A range of familial metabolic disorders of tyrosine catabolism is characterized by excretion of tyrosine and tyrosine catabolites in urine
- The defect in inherited tyrosine abnormalities is either a deficiency in tyrosine aminotransferase, resulting in tyrosinemia II, a deficiency of 4-hydroxyl–phenylpyruvic acid oxidase, leading to tyrosinoma type III or, more commonly a deficiency of fumarylacetoacetate (FFA)hydrolase, resulting in tyrosinemia I
- The absence of these enzymes results in abnormally high levels of tyrosine and, in some cases, increases in PHPPA and methionine
- The elevated tyrosine leads to liver damage, which may be fatal in infancy or to cirrhosis and liver cancer later in life
- The incidence of tyrosinemia I is approximately 1 of 100,000 births.

Diagnosis

- The disease is diagnosed by elevated tyrosine level using MS/MS coupled with a confirmatory test for an elevated level of the abnormal metabolite succinylacetone

Alkaptonuria

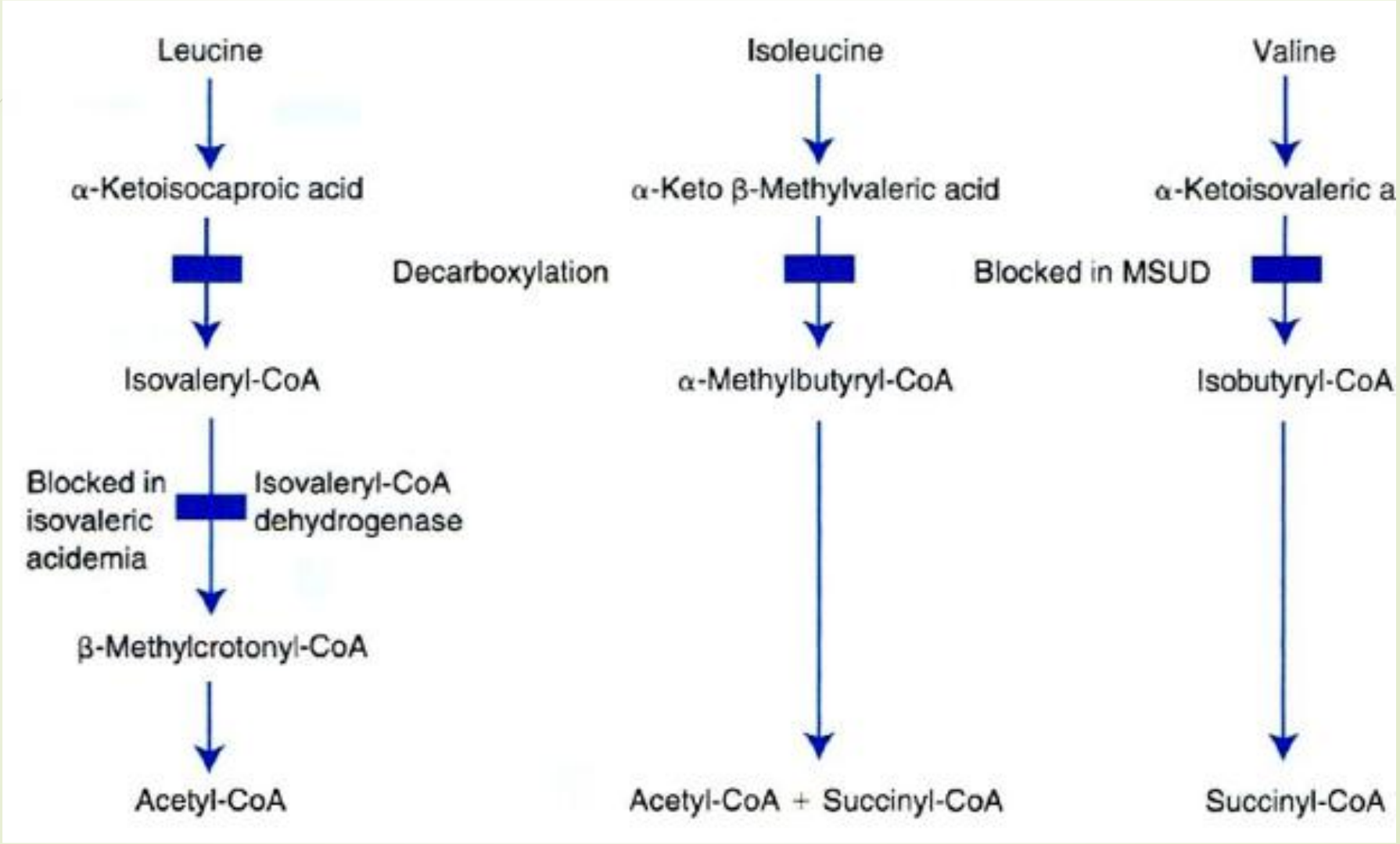
- This disorder is one of the original inborn errors of metabolism that showed a pattern of familial inheritance
- The biochemical defect in alkaptonuria is a lack of homogentisate oxidase in the tyrosine catabolic pathway
- This disorder occurs in about 1 of 250,000 births.
- of HGA in the urine, which oxidizes to produce a dark polymer.
- Alkaptonuria patients have no immediate problems but late in the disease, the high level of HGA, gradually accumulates in the connective tissue, causing generalized pigmentation of these tissues (ochronosis) and an arthritis-like degeneration

Maple syrup urine disease (MSUD)

- also called branched-chain ketoaciduria
- An autosomal recessive metabolic disorder affecting branched-chain amino acids. It is one type of organic acidemia
- MSUD is caused by a deficiency of the branched chain a-keto acid dehydrogenase complex (BCKDH), leading to a buildup of the branched-chain amino acids (leucine, isoleucine and valine) and their toxic by-products (ketoacids) in the blood, urine and cerebrospinal fluid (CSF).
- The disease is characterized in an infant by the characteristic maple syrup or burnt sugar odor of the urine, breath, and skin.

Maple syrup urine disease (MSUD)

- Typically infants with this inherited abnormality appear normal at birth but, by age 4-7 days, develop lethargy, vomiting, and signs of failure to thrive
- Central nervous system (CNS) symptoms follow including muscle rigidity, stupor, and respiratory irregularities
- If left untreated, the disease causes severe mental retardation, convulsions, acidosis, and hypoglycemia
- In the classic form of the disease, death usually occurs during the 1st year
- In less severe variants, the activity of the decarboxylase is approximately 25% of normal. Although this still results in a persistent elevation of the branched amino acids, the levels frequently can be controlled by limiting dietary protein intake


Leucine
α-Ketoisocaproic acid
Isovaleryl-CoA
Blocked in isovaleric acidemia
Isovaleryl-CoA dehydrogenase
β-Methylcrotonyl-CoA
Acetyl-CoA
Decarboxylation
Isoleucine
α-Keto β-Methylvaleric acid
α-Methylbutyryl-CoA
Acetyl-CoA + Succinyl-CoA
Valine
α-Ketoisovaleric a
Blocked in MSUD
Isobutyryl-CoA
Succinyl-CoA

Screening method

- A modified Guthrie test is commonly used for this neonatal screening.
- The metabolic inhibitor to B. subtilis, included in the growth media, is 4-azaleucine
- In a positive test for MSUD, an elevated level of leucine from a filter paper disk impregnated with the infant's blood will overcome the inhibitor and bacterial growth occurs.

Screening method

- Microfluorometric assay for branched-chain amino acids, using leucine dehydrogenase, can be used for mass screening
  - The filter paper specimen is treated with a solvent mixture of methanol and acetone to denature the hemoglobin
  - Leucine dehydrogenase is added to an aliquot of this sample extract. The fluorescence of the NADH produced in the subsequent reaction is measured at 450 nm, using an excitation wavelength of 360 nm.
  - A confirmed diagnosis is based on finding increased plasma and urinary levels of the three branched-chain amino acids and their ketoacids, with leucine being in highest concentration
  - A leucine level above 4 mg/dL is indicative of MSUD. The presence of alloisoleucine, an unusual metabolite of isoleucine is characteristic.
- Measurement of leucine and its metabolites is also possible using tandem mass spectrometry. MSUD can be diagnosed prenatally by measuring the decarboxylase enzyme concentration in cells cultured from amniotic fluid.

Isovaleric acidemia

- Isovaleric academia results from a deficiency of the enzyme isovaleryl-CoA dehydrogenase in the degradative pathway of leucine
- The resultant elevation of the glycine conjugate of isovaleric acid, isovalerylglycine, produces a characteristic “sweaty feet” odor.
- The abnormal organic acid levels can be identified by chromatography or MS/MS.

Homocystinuria

- Homocysteine is an intermediate amino acid in the synthesis of cysteine from methionine.
- The usual cause of the hereditary disease, homocystinuria, is an impaired activity of the enzyme cystathionine β-synthase, which results in elevated plasma and urine levels of the precursors homocysteine and methionine
- Newborns show no abnormalities, but physical defects develop gradually with age
- Associated clinical findings in the late childhood include thrombosis resulting from toxicity of homocysteine to the vascular endothelium, osteoporosis, dislocated lenses in the eye resulting from the lack of cysteine synthesis essential for collagen formation and mental retardation.

Screening test

- The enzyme cystathionine β -synthase requires vitamin B6 (pyridoxine) as its cofactor.
- There are two forms of the disease:
  - A vitamin B6-responsive form, in which treatment consists of therapeutic doses of vitamin B6
  - A vitamin B6-unresponsive form, in which the treatment is a diet low in methionine and high in cysteine
- The incidence of homocystinuria is approximately 1 of 200,000 births
- Screened in infants by Guthrie test using L-methionine sulfoximine as the metabolic inhibitor
  - Increased plasma methionine levels from affected infants will result in bacterial growth
  - A level of methionine greater than 2 mg/dL using an HPLC procedure confirms positive results on the screening test

Neonatal screening

- Alternately screening programs can use MS/MS to test for methionine
- Elevations in urinary homocystine can be detected by the cyanide-nitroprusside spot test
  - Cystine and homocystine reduced by sodium cyanide to their free thiol form, cysteine and homocysteine, which can then react with sodium nitroprusside to produce a red-purple color
  - Because cysteine also produces a positive result, the presence of homocysteine must be confirmed with a silver nitroprusside test. Silver nitrate reduces homocysteine but not cysteine, allowing only homocysteine to react with nitroprusside and produce a reddish color.
  - Cystine remains in the oxidized form, which does not react with sodium nitroprusside

Neonatal screening

- Elevations of homocysteine are of interest in the cardiovascular risk. It was found that approximately 50% of individual with untreated homocystinuria with significantly elevated levels of plasma homocysteine (200-300 µmol/L) had experienced a thromboembolic event before the age of 30.
- Mild homocysteine elevation (>15 µmol/L) occurs in 20-30% of patients with atherosclerotic disease
- In addition to cystathionine- synthase deficiency hyperhomocystinemia can be caused low folate concentration, vitamin B1 deficiency, decline in renal function, and genetic alteration of the enzyme, methylenetetrahydrofolate reductase (MTHFR), which converts homocysteine back to methionine.
- Although there is evidence of endothelial dysfunction in patients with elevated homocysteine levels, there is a disagreement with whether mild hyperhomocystinemia is a causative factor in the development of atherosclerotic disease or a consequence of the disease process

Argininosuccinic aciduria and Citrullinemia

- Results from inherited enzyme deficiencies in the urea cycle, arginine succinic aciduria results from the deficiency in argininosuccinic acid (ASA) lyase and a decrease in activity of ASA synthetase causes citrullinemia
- Symptoms include vomiting and high ammonia levels, and mental retardation is associated with some of the conditions
- MS/MS technology has allowed measurement of the affected metabolites.
- Citrulline is the diagnostic marker for both citrullinemia and argininosuccinic aciduria
- Citrulline is dramatically elevated in citrullinemia, while in argininosuccinic aciduria, the increase in citrulline is milder and increases in ornithine and arginine are seen in older infants

Cystinuria

- Caused by a defect in the amino acid transport system rather than a metabolic enzyme deficiency
- Normally amino acids are freely filtered by the glomerulus and then actively reabsorbed in the proximal renal tubules
- In cystinuria, there is 20-30-fold increase in the urinary excretion of cysteine as a result of genetic defect in the renal resorptive mechanism
- The transport mechanism is not specific for cysteine. Excretion of the other amino acids, lysine, arginine, and ornithine, is also significantly elevated as a result of deficient resorption.

Cystinuria

- Of the four, cysteine is relatively insoluble, when it reaches high levels in the urine, it tends to precipitatein the kidney tubules and form urinary calculi.
- The formation of cysteine calculi can be minimized by a high fluid intake and alkalinizing the urine, which makes cysteine relatively more soluble
- If this does not succeed, treatment with regular doses of penicillamine can be initiated
- cystinuria can be diagnosed by tesing the urine for cysteine using cyanide-nitroprusside, which produces a red-purple color on reaction with sulfhydryl groups.
- False-positive results as a result of homocystine must be ruled out.

CASE STUDY 8-1

A 13-month-old boy was admitted to a small, rural hospital.[1] He had been in a normal state of health until 10 days prior, at which time he developed an upper respiratory tract infection. He experienced increasing problems with his balance and became lethargic. These symptoms prompted his mother to seek medical attention at the hospital where pertinent laboratory results at admission showed a serum glucose level of 23 mg/dL and moderate ketones in the urine and serum. An intravenous solution of 5% dextrose was initiated to correct the low glucose level. Because the clinical picture resembled Reye's syndrome, the child was transferred to a medical center hospital for a definitive diagnosis. Laboratory results on admission to the medical center hospital appear in Case Study Table 8-1.1.

1. Campbell P. Case studies. J Med Tech 1985;2:9.

Questions

1. Which laboratory result can be useful in ruling out the diagnosis of Reye's syndrome?
2. What correlations can be made using the blood pH, PCO_2, serum glucose, and serum and urine ketone findings?
3. What other laboratory tests should be performed to verify a defect in protein metabolism?

CASE STUDY TABLE 8-1.1. ADMISSION LABORATORY RESULTS

HEMATOLOGY		URINALYSIS	
Hct	37%	Specific gravity	1.022
WBC	128 × 10^9/L	Protein	Trace
Bands	28%	Acetone	3+
Segmented	46%	Blood	1+
Lymphocytes	21%		
Monocytes	5%		
CHEMISTRY (REFERENCE RANGE)			
Glucose	133 mg/dL (65–105)	Alk phos	129 U/L (20–70)
BUN	21 mg/dL (7–18)	AST	154 U/L (10–30)
Na	136 mmol/L (136–145)	ALT	133 U/L (8–20)
K	4.3 mmol/L (3.6–5.1)	CK	36 U/L (25–90)
TCO_2	10 mmol/L (23–29)	LD	119 U/L (45–90)
Cl	112 mmol/L (98–106)	Ketone	Moderate
NH_3	48 µmol/L (40–80)		

ARTERIAL BLOOD GASES	
pH	7.17
PCO_2	23 mm Hg
PO_2	90 mm Hg

Proteins

- General characteristics
  - Proteins are an essential class of compounds comprising 50-70% of the cell's dry weight.
  - Proteins are found in all cells of the body as well as in all fluids, secretions and excretions
- Molecular Size
  - Biological active proteins are macromolecules that range in molecular weight from approximately 6000 for insulin to several million for some structural proteins.
- Structure
  - All proteins comprise covalently linked polymers of amino acids.
  - The carboxy group of one amino acid combines with the amino group of another amino acid by peptide bond
  - In human serum, proteins average about 100-150 amino acids in the polypeptide chain
  - The conformation of a protein is determined by interaction between a polypeptide and its aqueous environment

Protein structure and characteristics

- The primary structure is crucial for the function and molecular characteristics of the protein.
- Denaturation can be caused by heat, hydrolysis by strong acid or alkali, enzymatic action, exposure to urea or other substances, or exposure to ultraviolet light
- **The nitrogen content** of serum protein varies somewhat, the average is approximately 16%. This characteristic is used in one method of total protein measurement
- Each protein has its own **isoelectric point (pI)** which is the pH at which a protein has no net charge which help in isolation of proteins by electrophoresis
- Proteins are antigenic
- Protein form colloidal solution or micelles because they are charged produces an envelope of water around it which make possible to precipitate using different concentrations of salt and nonpolar solvents.

Proteins

- Classification of proteins :
  - Simple proteins: contain peptide chains that on hydrolysis yield only amino acids (can be globular or fibrous)
  - Conjugated proteins: comprise a protein (apoprotein) and a a nonprotein moiety (prosthetic group)
    - The prosthetic group may be lipid, carbohydrate. Porphyrins or metal
- Function of protein
  - Plasma proteins and tissue proteins share the same amino acid pool and so important in tissue nutrition.
  - Distribution of water among the compartments of the body by osmotic forse of plasma proteins
  - They act as buffers within the plasma and interstitial tissue

General function of proteins

- Many plasma proteins functions as specific transporters of metabolic substances as thyroxine-binding globulin and albumin
- Several proteins are glycoproteins which function to distinguish which cells are native and which are foreign to the body
- Many cellular proteins act as receptors for hormones.
- Certain hormones (e.g. growth hormone and adrenocorticotropic hormone (ACTH)) are themselves proteins
- Proteins also serve a structural role as collagen
- Some proteins (enzymes)

Plasma proteins
Prealbumin (transthyretin)

- It is rarely observed as a distinct band on routine cellulose acetate electrophoresis patterns of serum, although it can be exhibited by high-resolution electrophoresis (HRE) or immunoelectrophoresis
- Prealbumin is used in the body to transport thyroxine and triiodothyronine, in addition to the transportation of retinol (vitamin A)
- Prealbumin is **decreased** in hepatic damage, acute phase inflammatory response, and tissue necrosis
- A low prealbumin level is a sensitive marker of poor protein nutritional status which results in a decrease in the level of the proteins originating in the liver, including prealbumin (short half life, 2 days)
- Prealbumin is i**ncreased** in patients receiving steroids, in alcoholism, and in chronic renal failure

Albumin

- Albumin is the protein present in highest concentration in the serum that is synthesized in the liver.
- Because of its high concentration in blood, albumin is responsible for nearly 80% of osmotic pressure
- Albumin binds bilirubin, salicylic acid, fatty acids, calcium, magnesium ions, cortisol and some drugs. This characteristic is also exhibited with certain dyes, providing a method for the quantitation of albumin

Albumin

- Decreased conc. of serum albumin may be caused by the following:
  - **An inadequate source** of amino acids (malnutrition and muscle-wasting disease)
  - **Liver disease**, resulting in the ability of hepatocytes to synthesize albumin. The increase in globulins that occurs in the early cirrhosis will balance the loss in albumin to give a total protein concentration within acceptable limits. The decline in serum albumin is insignificant in viral hepatitis.
  - **Gastrointestinal loss** as interstitial fluid leaks out in inflammation and disease of the intestinal mucosa
  - Loss in the urine in **renal disease**.
  - **Analbuminemia:** the absence of albumin because of genetic origin resulting from an autosomal recessive trait
  - **Bisalbuminemia:** the presence of albumin that has unusual molecular characteristics demonstrated by the presence of two albumin bands instead of the single band usually seen by electrophoresis

Albumin

- Increased serum levels are seen in **Dehydration**
- Administering fluids to treat the dehydration will decrease serum albumin levels back to normal.

- The earliest method for its determination involved the salting out of the globulins with sodium sulfate leaving the albumin in solution
- The albumin was then determined by the biuret color development. The method commonly used today involves a dye binding and shift in color when a dye is bound by albumin
- When more information about proteins is needed, an electrophoretic pattern is obtained, and albumin is calculated as percentage of the total protein (usually, approximately 60%)
- At birth, the reference value for serum albumin averages 39g/L. the concentration falls to 28.4 g/L at about 9 months and then begins to increase slowly until adult values of 35-55 g/L are reached.

Globulins
a1-Antitrypsin

- Its main function to neutralize trypsin –like enzymes (as elastase) a1-Antitrypsin is a major component (90%) of the fraction of serum protein that migrates electrophoretically immediately following albumin.
- A deficiency of a1-Antitrypsin is associated with severe, degenerative emphysematous pulmonary disease due to proteolytic activity of proteases from leukocytes in the lung during periods of inflammation

- **Juvenile hepatic cirrhosis** is also correlative disease in a1-Antitrypsin deficiency. The protein is synthesized but not released from the hepatocyte.
- Increased levels of a1-Antitrypsin are seen in inflammatory reactions, pregnancy and contraceptive use

a1-Antitrypsin

- The discovery of abnormal a1-Antitrypsin levels is most often made by the lack of an a1-globulin band on protein electrophoresis.
- The discovery is followed with one of the quantitative methods. A widely used method is radial immunodiffusion
- Immunonephelometric assays by automated instrumentation are also available. Phenotyping can be accomplished by immunofixation

a1-Fetoprotein

- a1-fetoprotein (AFP) is synthesized initially by the fetal yolk sac and then by the parenchymal cells of the liver
- It peaks in the fetus at about 13 weeks of gestation (3 mg/ml) and recedes at 34 weeks gestation. At birth, it recedes rapidly to adult concentration, which are normally very low
- The methods commonly used for AFP determinations are radioimmunoassay and enzyme labeled immunoassay
- It has been proposed that the protein protects the fetus from immunolytic attack by its mother, modulates cell growth transport compounds such as steroids and is required for the functional development of the female reproductive system
- AFP is detectable in the maternal blood up to month 7 or 8 of pregnancy (transmitted across the placenta). AFP in maternal serum is a screening test

a1-Fetoprotein

- Elevated AFP level include:
  - neural tube defects, atresia of the gastrointestinal tract and fetal distress in general. Its use in determining neural tube defects before term is an important reason for its assay.
  - It is also increased in ataxia-telangiectasia, tyrosinosis, and hemolytic disease of the newborn
  - maternal serum AFP is also increased in the presence of twins.
- Low levels of maternal AFP indicate an increased risk for Down's syndrome and trisomy 18
- are also affected by maternal weight, which reflects blood volume (inverse relationship), race (10% higher in African Americans), and diabetes (lowered value)
- Serum levels of AFP can also be used as a tumor marker (high in hepatocellular carcinoma (80%) and certain gonadal tumors in adults

Ceruloplasmin

- Ceruloplasmin is copper-containing a2-glycoprotein that has enzymatic activities (ie. Copper oxidase, histaminase and ferrous oxidase)
- It is synthesized in the liver. 90% or more of total serum copper is found in ceruloplasmin
- The early analytic method of ceruloplasmin determination was based on its copper oxidative capacity
- Most assays today use immunochemical methods, including radial immunodiffusion and nephelometry
- Low concentrations of ceruloplasmin at birth gradually increase to adult levels and slowly continue to rise with age. Adult females have higher concentrations than males and pregnancy, inflammatory processes, malignancies, oral estrogen and contraceptives cause an increased serum concentration.

Ceruloplasmin

- Certain diseases or disorders are associated with low serum concentrations. In Wilsons disease, an autosomal recessive inherited disease, the levels are typically low (0.1 g/L)
- Total serum copper is decreased, but the direct reacting fraction is elevated and the urinary excretion of copper is increased
- The copper is deposited in the skin, liver and brain, resulting in hepatic cirrhosis and neurological damage. Copper also deposits in the cornea, producing the characteristic Kayser-Fleischer rings
- Low ceruloplasmin is also seen in malnutrition, malabsorption, severe liver disease, nephrotic syndrome, and Menke's syndrome (kinky hair disease), in which a decreased absorption of copper results in a decrease in ceruloplasmin

Transferrin (Siderophillin)

- Transferrin, a glycoprotein, is synthesized primarily by the liver.
- Transferrin is the major component of the β-globulin fraction and appears as distinct band on high-resolution serum protein electrophoresis
- Genetic variation of transferrin has been demonstrated by electrophoresis on polyacrylamide gel
- Precise and accurate analytic methods used for the quantitation of transferrin include immunodiffusion and immunonephelometry
- The major fractions of transferrin are the transport of iron and the prevention of loss of iron through the kidney and deposition in the tissue during temporary increases in absorbed iron or free iron. Transferrin transports iron to its storage sites (ferritin) and to bone marrow that synthesize hemoglobin

Transferrin (Siderophillin)

- The most common form of anemia is **iron deficiency** anemia where transferrin in serum is normal or increased.
- A decreased transferrin level reflects an overall decrease in the synthesis of protein (as seen in **liver disease** or **malnutrition** and **protein-losing disorders** such as nephrotic syndrome.
- Transferrin, **a negative acute phase protein**, is also decreased in inflammation. A deficiency of plasma transferrin may result in the accumulation of iron in apoferritin
- Patients with hereditary transferrin deficiencies have been shown to have significant hypochromic anemia. An increased of iron bound to transferrin is found in hereditary disorder of iron metabolism, hemochromatosis, in which excess iron is deposited in the tissue, especially the liver and the pancreas. This disorder is associated with bronze skin, cirrhosis, diabetes mellitus, and low plasma transferrin levels

Lipoprotein

- They are complexes of proteins and lipids whose function is to transport cholesterol, triglycerides, and phospholipids in the blood

- Lipoproteins are subclassified according to the apoprotein and specific lipid content
  - On high-resolution serum protein electrophoresis, high-density lipoproteins (HDL) migrate between the albumin and the a1-globulin zone
  - Very-low-density lipoprotein (VLDL) migrate at the beginning of the β-globulin fraction (pre-β)
  - The low density lipoproteins (LDL) appear as a separate band in the β-globulin region

Fibrinogen

- One of the largest proteins in blood plasma. It is synthesized in the liver and it is classified as a glycoprotein because it has considerable carbohydrate content.
- The function of fibrinogen is to form fibrin clot when activated by thrombin, therefore, fibrinogen is virtually all removed in the clotting process and is not seen in serum.
- Fibrinogen customarily has been determined as clot- table protein.
- Fibrinogen concentration is proportional to the time required to form a clot after the addition of thrombin to citrated plasma

Fibrinogen

- Degradation products of fibrinogen and fibrin are determined by immunoassay methods such as immunodiffussion, nephelometry and radioimmunoassay
- Fibrinogen is one of the acute phase reactants (significantly increased in plasma during acute phase of inflammatory process)
- Fibrinogen levels also rise with pregnancy and the use of birth control pills
- Decreased values generally reflect extensive coagulation, during which the fibrinogen is consumed

Troponin

- Troponin is complex of three proteins that bind to the film filaments of striated muscle (cardiac and skeletal) but are not present in smooth muscles
- The complex consists of troponin T, troponin I, and troponin C
- Cardiac troponin T in serum begins to rise within 3-4 hours following the onset of myocardial damage, peak in 10-24 hours, and remain elevated for 10-14 days following AMI
- Cardiac troponin I is also highly specific for myocardial tissue
- Because cTnI, like cTnT does not normally circulate in the blood and it is 13 times more abundant in the myocardium than CK-MB on a weight basis, cTnI is a very sensitive indicator of even a minor amount of cardiac necrosis

Total protein abnormalities Hypoproteinemia

- Occurs in any condition where a negative nitrogen balance exists
- Plasma proteins is excessive loss as in renal disease (ie. Nephrotic syndrome) leakage into the GIT in inflammation of the digestive system and in loss of blood in open wounds, internal bleeding, or extensive burns
- Decreased intake either because of deficiency of protein in diet (malnutrition) or through intestinal malabsorption due to structural damage
- A decrease in serum proteins as a result of decreased synthesis is also seen in liver disease
- Hypoproteinemia may result from accelerated catabolism of proteins, such as in burns, trauma, or other injuries

Hyperproteinemia

- An elevation of all protein fractions is observed is dehydration which may result from a variety of conditions, including vomiting, diarrhea, excessive sweating, diabetic acidosis, and hypoaldosteronism
- Hyperproteinemia may be result of excessive production, primarily of the γ-globulins
- The appearance of monoclonal protein or paraprotein in the serum and often in the urine as well

Hyperproteinemia

- The most common disorder is multiple myeloma, in which the neoplastic plasma cells proliferate in the bone marrow
- The paraproteins in this case is usually IgG and IgA. IgD and IgE paraproteins rarely occur. Paraproteins in multiple myeloma may reach a serum concentration of several grams per deciliter
- Not all paraproteins are associated with multiple myeloma. IgM paraprotein is often found in pateints with Waldenstorms macroglobulinemia, a more benign condition.
- Many disorders including chronic inflammatory states, collagen vascular disorders, and other neoplasms, may be associated with paraproteins
- Polyclonal increases in immunoglobulins are seen in the serum and urine in many chronic diseases

TABLE 8-5. PROTEIN LEVELS IN SELECTED DISEASE STATES

TOTAL PROTEIN	ALBUMIN	GLOBULIN	DISEASE
N, ↓	↓	↑	Hepatic Damage • Cirrhosis β-γ bridging • Hepatitis ↑ γ-globulins • Obstructive jaundice ↑ α_2-, β-globulins Burns, Trauma Infections • Acute ↑ α_1-, α_2-globulins • Chronic ↑ α_1-, α_2-, γ-globulins
↓	↓	N	Malabsorption Inadequate Diet Nephrotic Syndrome ↑ α_2-, β-globulins; ↓ γ-globulins
↓	N	↓	Immunodeficiency Syndromes
↓	↓	↓	Salt Retention Syndrome
↑	↑	↑	Dehydration
↑	N	↑	Multiple Myeloma Monoclonal and Polyclonal Gammopathies

↑ = increased; ↓ = decreased; N = normal levels.

CASE STUDY 8.2

Immediately following the birth of a baby girl, the attending physician requested a protein electrophoretic examination of the mother's serum. This was done on a sample that was obtained on the mother's admission to the hospital the previous day. An electrophoretic examination was also performed on the cord-blood specimen. Laboratory reports are shown in Case Study Table 8-2.1.

The appearance of the mother's electrophoretic pattern was within that expected for a healthy person. The electrophoretic pattern of the cord-blood serum resembled the one shown in Figure 8-13C.

Questions

1. What protein fraction(s) is/are abnormal in the mother's serum and the cord-blood serum?
2. An abnormality in this/these fraction(s) is/are most often associated with what disease?
3. What other test(s) may be done to confirm this abnormality?

CASE STUDY TABLE 8-2.1 ELECTROPHORESIS (VALUES g/dL)

	ADULT REFERENCE VALUES	MOTHER'S SERUM	CORD BLOOD
Albumin	3.5–5.0	4.2	3.3
α_1-Globulins	0.1–0.4	0.3	0.0
α_2-Globulins	0.3–0.8	1.2	0.4
β-Globulins	0.6–1.1	1.3	0.7
γ-Globulins	0.5–1.7	1.3	1.0

CASE STUDY 8-3

A 76-year-old woman was admitted to the hospital with gangrene of her right toe. She was disoriented and had difficulty finding the right words to express herself. On evaluation, it was revealed she lived alone and was responsible for her own cooking. A daughter who lived in the area said her mother was a poor eater, even with much encouragement. An ECG, performed on admission, showed possible ectopic rhythm with occasional premature supraventricular contractions. The cardiologist suspected a possible inferior myocardial infarction of undetermined age. Lab results are shown in Case Study Table 8-3.1.

Questions

1. In this patient, what is the clinical value of the troponin I measurements?
2. What is a possible explanation for the elevated myoglobin?
3. What condition is indicated by the low prealbumin value?

CASE STUDY TABLE 8-3.1 LABORATORY RESULTS

Day 1		
CK-total	187 U/L	(40–325)
CK-MB Mass	6 µg/L	(<8)
Troponin I	16.3 µg/L	(0–2)
Prealbumin	15 mg/dL	(17–42)
Albumin	2.7 g/dL	(3.7–4.9)
Repeat (5 Hours Later)		
CK-total	180 U/L	
CK-MB mass	5.4 µg/L	
Troponin I	17.5 µg/L	
Day 2		
CK-total	177 U/L	
CK-MB mass	4.5 µg/L	
Troponin I	13.7 µg/L	
Myoglobin	<500 µg/L	(<76)

CASE STUDY 8-5

A 45-year-old man was undergoing continuing evaluation of possible recurrence of a plasmacytoma that had originally presented with a compression fracture of a vertebra. He had been treated with local radiation and chemotherapy. His serum protein electrophoresis showed normal amounts of albumin, α_1, α_2, and β fractions. The γ fraction demonstrated a slight monoclonal band in the fast γ region (close to β). Protein electrophoresis of concentrated urine showed a single monoclonal band that migrated slightly less than the serum band. *(Case 8-5 courtesy of Dr. R. McPherson, Chairman, Clinical Pathology, Medical College of Virginia Hospitals, Virginia Commonwealth University Health System)*

Questions

1. Does the presence of the monoclonal band in the serum indicate the recurrence of the patient's tumor?
2. What further information is obtained from a urine protein electrophoresis?
3. What other test is needed to confirm the type of urinary protein?

CASE STUDY 8-8

A 36-year-old woman complained of intermittent blurred vision and numbness and weakness in her left leg that had persisted for more than three weeks. On examination, vertical nystagmus (involuntary back-and-forth or circular movements of eyes) was noted on upward gaze. CSF was drawn and the specimen was clear and colorless with normal cell count. The CSF total protein level was 49 mg/dL with an IgG of 8.1 mg/dL. Electrophoresis of the patient's serum and CSF revealed the following pattern:

Questions

1. What is the significance of the protein bands indicated by the arrows?
2. What conditions would produce this type of protein electrophoresis pattern?
3. What other tests would be helpful in the investigation of this patient's diagnosis?
4. What laboratory test can be useful for monitoring the course of this patient's condition?