Demonstrated by

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1434-1435H

Practical Lab CLS333

Part# 2

***Definitions:**

-Hemolyzed sample:

is the rupturing of erythrocytes (red blood cells) and the release of their contents (cytoplasm) into surrounding fluid (*e.g.*, serum or plasma).

-Icteric sample:

serum or plasma varies in color from dark to bright yellow, rather than the normal color. Icterus may affect certain determinations and can seen in hyperbilirubinemia

-Lipemic sample:

Turbid, cloudy or milky serum (lipemic serum) may be produced by the presence of fatty substances (lipids) in the blood .it recommend that patients fast 12-16 hours before a blood specimen is obtained.

Lab 5:
Triglycerides

Quantitative determination of the TG in serum or plasma

***Lipid:**

The major lipids of the body (TG,FA, cholesterol, phospholipids and glycolipids)

*They serve as:

-Source of fuel.

-Important component of cell membranes and cells structures.

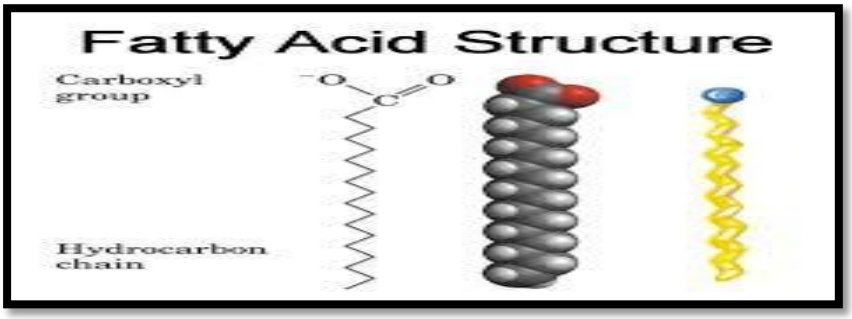
-They provide stability to the cell membrane and allow for transmembrane transport.

-They are transported through the blood stream in the form of lipoprotein

***Fatty Acids:**

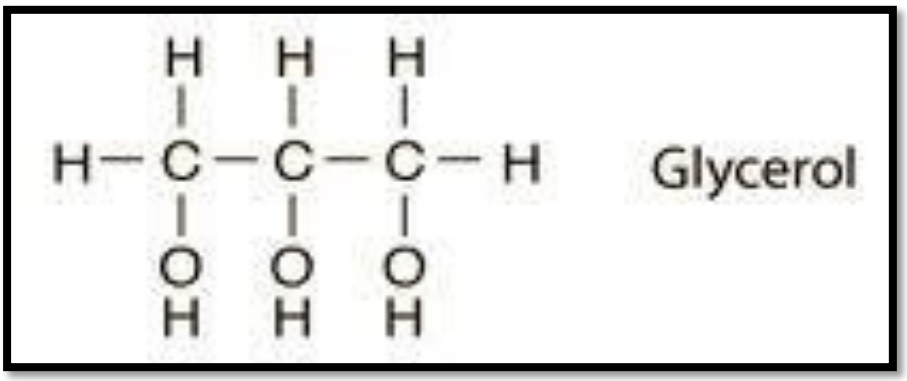
The lipid building blocks.

Fatty acids are composed of a chain of methylene groups with a Carboxyl functional group at one end.



***Glycerol:**

Glycerol is a tri-hydric alcohol (containing three -OH hydroxyl groups) that can combine with up to 3 fatty acids to form mono-glycerides, di-glycerides, and tri-glycerides

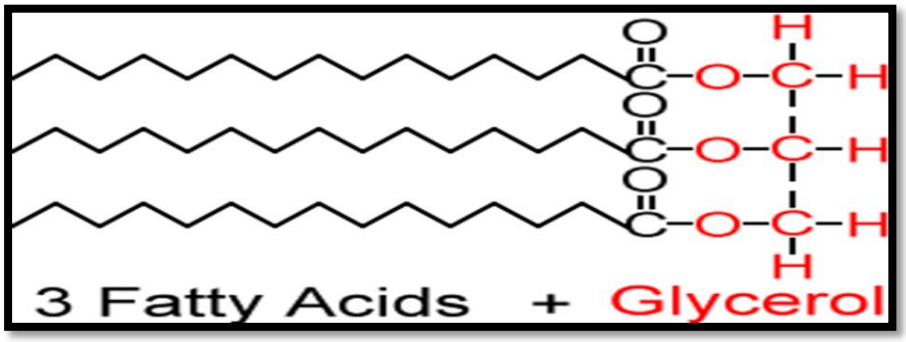


***Triglyceride:**

TG consist Glycerol and 3 FA, it's major type of lipid used for energy storage.

It's found in droplets within the cytoplasm

It's non-polar and relatively insoluble



***source:**

The source of TG in the body can be either:

dietary or synthesized in liver and other tissues.

TG molecules allow the body to compactly store long carbon chains(FA) for energy that can be used during fasting states between meals.

***Clinical significance:**

Elevated levels of both cholesterol and triglycerides indicate to:

-Atherosclerotic disease

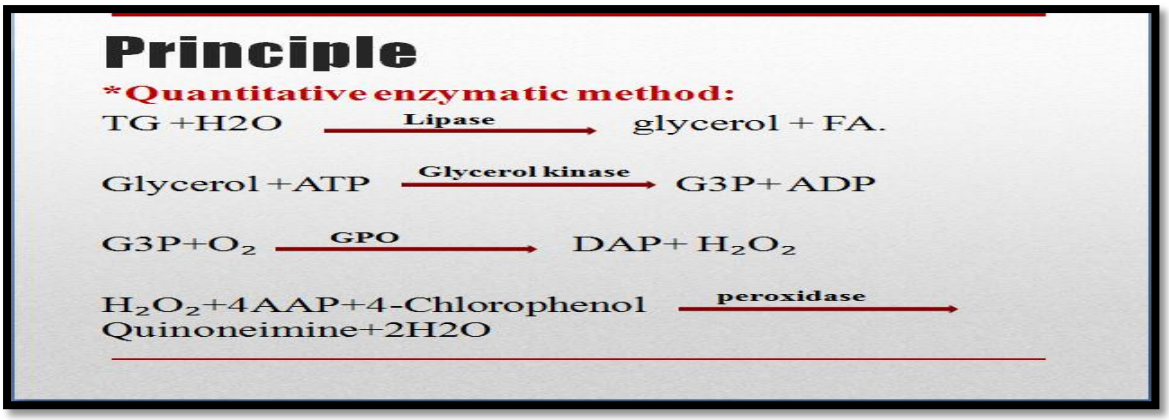
-hyperlipidemia→can be inherited traits or they can be secondary to a variety of disorders including DM, nephrosis, biliary obstruction and metabolic disorders associated with endocrine disturbances

Usually, TG conc. is requested either in combination with total cholesterol or as part of lipid panel examination (cholesterol, TG, HDL, LDL) To estimate the degree of risk for:

-hyperlipoproteinemia

-coronary artery disease

-Heart disease and stroke.



*AAP= aminoantipyrine

*GPO=glycerol-3-phosphate oxidase

The amount of the dye formed is directly proportional to the conc. of TG in the sample

***Specimen:**

Fresh, non-hemolyzed serum(Fasting better)

TG in serum appears stable for 3 days when stored at 2-8 °c

Procedure

	Balnk	Std	Test
WR-ml	1	1	1
Std-µl	---	10	---
Sample-µl	---	---	10
Mix and incubate at 37C for 10 min then read A at505 nm against blank			
Final color is stable for 30 min at RT			

Calculation

$$\frac{A\,test}{A\,Std} \times Conc.of\,std(200mg/dl) = TG\ in\ test\ (mg/dl)$$

***Normal Range:**

According age more than those values indicate hyperlipidemia

0 - 29 years ..... 10 - 140 mg/dl

30 - 39 years ..... 10 - 150 mg/dl (memorize this only)

40 - 49 years ..... 10 - 160 mg/dl

50 - 59 years ..... 10 - 190 mg/dl

***Interferences:**

Some lab mistakes lead to falsely elevation of TG level:

Glycerol in rubber stoppers or in contaminated glassware.

-Lipemic or grossly icteric

-Samples with gross hemolysis or high bilirubin values

-A number of drugs and substances affect the measurement of triglycerides

***Limitations:**

This reagent is linear up to 1000 mg/dl Triglycerides.

Samples with values above 1000 mg/dl should be diluted with water, re-assayed and the results to be multiplied by 2

Lab 6

Bilirubin

Quantitative determination of bilirubin in serum using a modified Malloy-Evelyn colorimetric/Endpoint procedure

Bilirubin is the yellow breakdown product of normal haeme catabolism. Haeme is found in haemoglobin of red blood cells. Bilirubin is excreted in bile and urine, and elevated levels may indicate certain diseases.

Bilirubin can be "conjugated" with a molecule of glucuronic acid which makes it soluble in water . This is an example of glucuronidation.

Unconjugated (in Direct)

Erythrocytes (red blood cells) generated in the bone marrow are disposed of in the spleen when they get old or damaged. This releases hemoglobin, which is broken down to heme as the globin parts are turned into amino acids. The heme is then turned into unconjugated bilirubin in the reticuloendothelial cells of the spleen. This unconjugated bilirubin is not soluble in water, due to intramolecular hydrogen bonding. It is then bound to albumin and sent to the liver.

Conjugated (Direct)

In the liver, bilirubin is conjugated with glucuronic acid by the enzyme glucuronyltransferase, making it soluble in water. Much of it goes into the bile and thus out into the small intestine. Though most bile acid is

re-sorbed in the terminal ileum to participate in enterohepatic circulation, conjugated bilirubin is not absorbed and instead passes into the colon.

*The reaction:

The measurement of direct bilirubin depends on its reaction with diazosulfanilic acid to create azobilirubin. However, unconjugated bilirubin also reacts slowly with diazosulfanilic acid, so that we add methanol to accelerate the reaction.

*Clinical significance:

-An increase in bilirubin conc. Is called jaundice.

High level of direct type means that the bile is not being properly excreted.

-High levels of indirect mean more Hb is being damaged.

*Elevation of total bilirubin occur due to:

Excessive hemolysis of RBC

Liver diseases e.g. hepatitis &cirrhosis

Obstruction of biliary tract e.g. gall stones

-Both conjugated (direct) and unconjugated (indirect) bilirubin are increased in hepatitis.

-The relative proportion of the conjugated fraction increases with progression of the disease until eventually the liver loses its ability to carry out the conjugation reaction

*Principle:

Bilirubin in the serum is coupled with diazotized sulfanilic acid to form azobilirubin.

The intensity of the purple color that is formed is proportional to the bilirubin concentration in the serum.

Bilirubin glucuronide(the conjugated or direct bilirubin) reacts with the diazo reagent in aqueous solution to form a colored diazo compound within 1 minute.

This is the calculation of Direct Bilirubin

The subsequent addition of methanol accelerates the reaction .of unconjugated bilirubin in the serum, and a value of total bilirubin is obtained after letting the sample stand for 5 minutes.

*This is the calculation of Total Bilirubin

The total bilirubin value represents the sum of the bilirubin glucoronide (direct) and the unconjugated (indirect) bilirubin.

The color formed in the reaction is measured photometrical at 540/ 546 nm

***Specimen:**

Serum

while processing samples, protect from direct light as loss of bilirubin may occur. Bilirubin in serum is reportedly stable for 4- 7 days, when stored in the dark at 2-8°C

Procedure #2

	Direct bilirubin		Total bilirubin	
	Test Blank	Test	Test Blank	Test
Sulfanilic Acid Rgt-µl	1000	1000	500	500
Sodium Nitrate Rgt-µl	---	20	---	20
Mix and let stand for at least 1 min				
Sample	50	50	50	50
After exactly 1min. read the absorbance of Test and Test Blank (of Direct bilirubin only) at 546 nm against DW				
Methanol	---	---	500	500
Mix and let stand for 5 min. at RT and read A of test and test Blank (Total Bilirubin) at 546 nm against DW				

Calculation

USING FACTOR(25)

1-Direct Bilirubin

(A. Test — A. Test Blank) x 25 = Direct Bilirubin (mg/dL).

2.-Total Bilirubin

(A Test — A Test Blank) x 25 = Total Bilirubin (mg/dL).

***Normal Range:**

Direct……up to 0.5 mg/dl

Total……..up to 1.0 mg/dl

***Limitation:**

1-Hemolysis interferes with the reaction to give falsely decreased values

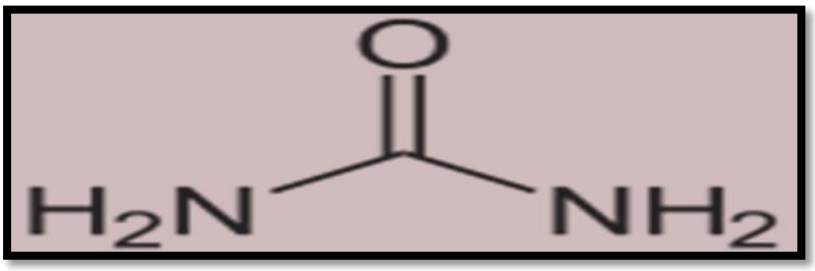
2-severe lipemia may cause loss of precision.

Lab 7

Urea

Urea:

Urea or carb-amide is an organic compound with the chemical formula (NH2)2CO. The molecule has two amine (-NH2) groups joined by a carbonyl (C=O) functional group.



Urea is synthesized in the liver as a by-product of the de-amination of amino acids so it is the main end product of protein metabolism in the body.

Its elimination in the urine represents the major route for nitrogen excretion (cleared from the blood by the kidney into the urine).

***Clinical significance:**

*Elevated urea seen:

-As a result of a high protein diet

-Increased protein catabolism

-After gastrointestinal hemorrhage

-Mild dehydration

-Shock and heart failure

-Treatment with gluco-cortcoids(pre-renal uremia).

-Diseases that compromise the function of the kidney often lead to increased blood levels of urea, as measured by the blood urea nitrogen (BUN) test

*Low urea level:(Not common)

They can be seen in severe liver disease or malnutrition but are not used to diagnose or monitor these conditions. Low urea levels are also seen in normal pregnancy.

The usefulness of urea as an indicator of renal function is limited by:

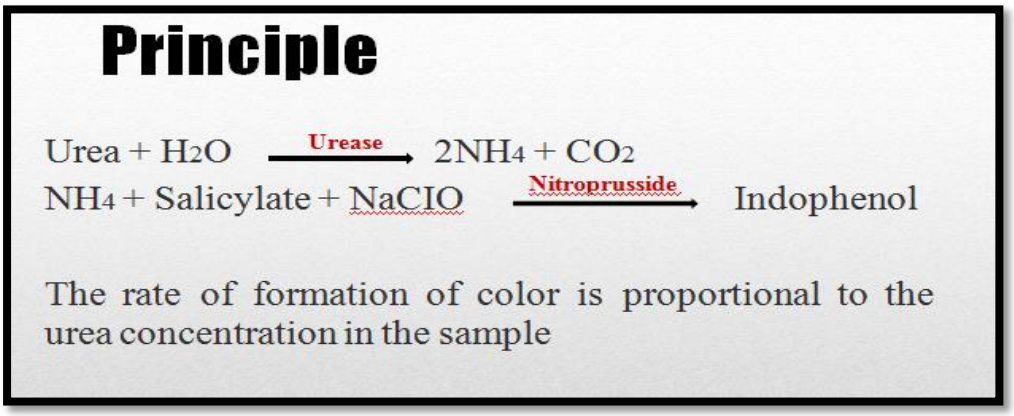
The variability of its plasma conc. As a result of non-renal factors

So, we can't depend on a single test

***Specimen:**

Serum or plasma (heparinized sample is recommended)

Urine, dilute urine 1/50 with DW before measurement



Procedure

	Blank	Std	sample
Rgt.A -ml	1	1	1
Std- µl	---	10	---
Sample-µl	---	---	10
Mix & incubate for 10 min at RT OR 5min at 37 C			
Rgt.B-ml	1	1	1
Mix & incubate for 10 min at RT OR 5 min at 37 C			
Read A at 600nm against blank and color will stable for 2hrs			

Calculation

$$\frac{A\ test}{A\ Std} \times conc.\ of\ Std(50mg/dl) = conc\ of\ urea\ (mg/dl)$$

***Normal Range for adult:**

15-39 mg/dl

Neonatal has lower urea than adult

Over 60 years will be higher

Conc. of urea tend to be slightly higher in males than females

***Limitation:**

-Lipemia & high bilirubin will not interfere.

-Hemolysis with elevated ammonia will interfere

-Some drugs may interfere

Lab 8:

Creatinine

Quantitative determination ofcreatinine in serum or heparinized plasma or urine by a colorimetric Kinetic method

Creatine:

Most of it is found in muscle tissues where it is present as creatine phosphate and serves as a high energy storage reservoir for conversion to ATP.

Independent of diet, serum creatinine concentrations depend almost entirely upon its excretion rate by the kidneys. For this reason its elevation is highly specific for kidney diseases

Creatinine:

Is an anhydride of creatine, is a waste product excerted by the kidneys &it's is an important part of muscle.

Level of its vary according to a person's size and muscle mass.

Females usually have a lower creatinine than males.

Three types to measure glomerular Filtration Rate(GFR):

A)Blood creatinine level.

B)Creatinine clearance test (Levels of 24-hour urine creatinine are evaluated with blood levels as part of a creatinine clearance test.

C)Blood-urea nitrogen: creatinine ratio

***Clinical significance:**

Elevate creatinine level may be indicative of renal problems.

Low blood levels of creatinine are not common

Principle

Creatinine + picric acid $\xrightarrow{\text{Alkali}}$ creatinine-picrate complex
Yellow-Orange

The rate of formation of color is proportional to the creatinine concentration in the sample.

***specimen:**

1. Serum, heparinized plasma or urine (dilute 50 times) i.e. 1ml urine + 49 ml water.

Procedure

	Std	Test
W.R -ml	1	1
Prewarm at 25c,or 37c for 2 minutes and add:		
Std-µl	100	---
Sample-µl	---	100
After exactly 20 seconds read and record absorbance Al against D.W at 505 nm. At exactly 60 sec after the Al, read and record the absorbance A2 and determine ΔA.		

Calculation

$\frac{\Delta A \text{ sample}}{\Delta A \text{ Std}}$ X conc. Of Std 1.5mg/dl= Cr (mg/dl)

Normal Range:

Serum

Male: 0.6-1.2 mg/dl

Female: 0.5-1.0 mg/dl

***Limitation:**

-Albumin at a concentration of 10.0 g/dl contributes 0.2 mg/dl to the creatinine value.

-Creatinine level will elevate with:

moderate hemolysis (0.2 g/dl Hgb) sample

grossly icteric sample

lipemic sample

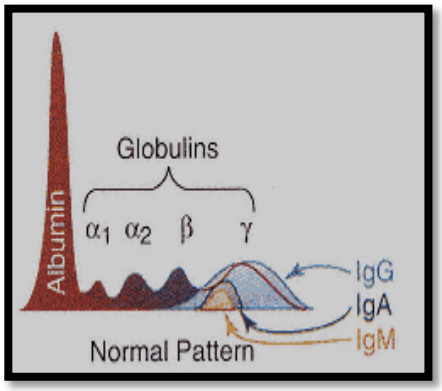
-Acetoacetate above 10 mg/dl will interfere with the results

*Electrophoresis technique use to demonstrate protein classification as apeak

1st peak is Albumin

Then alpha1-alpha2-Beta-Gamma

Any abnormality in the peak will show a disease.



Electrophoresis patterns for protein:

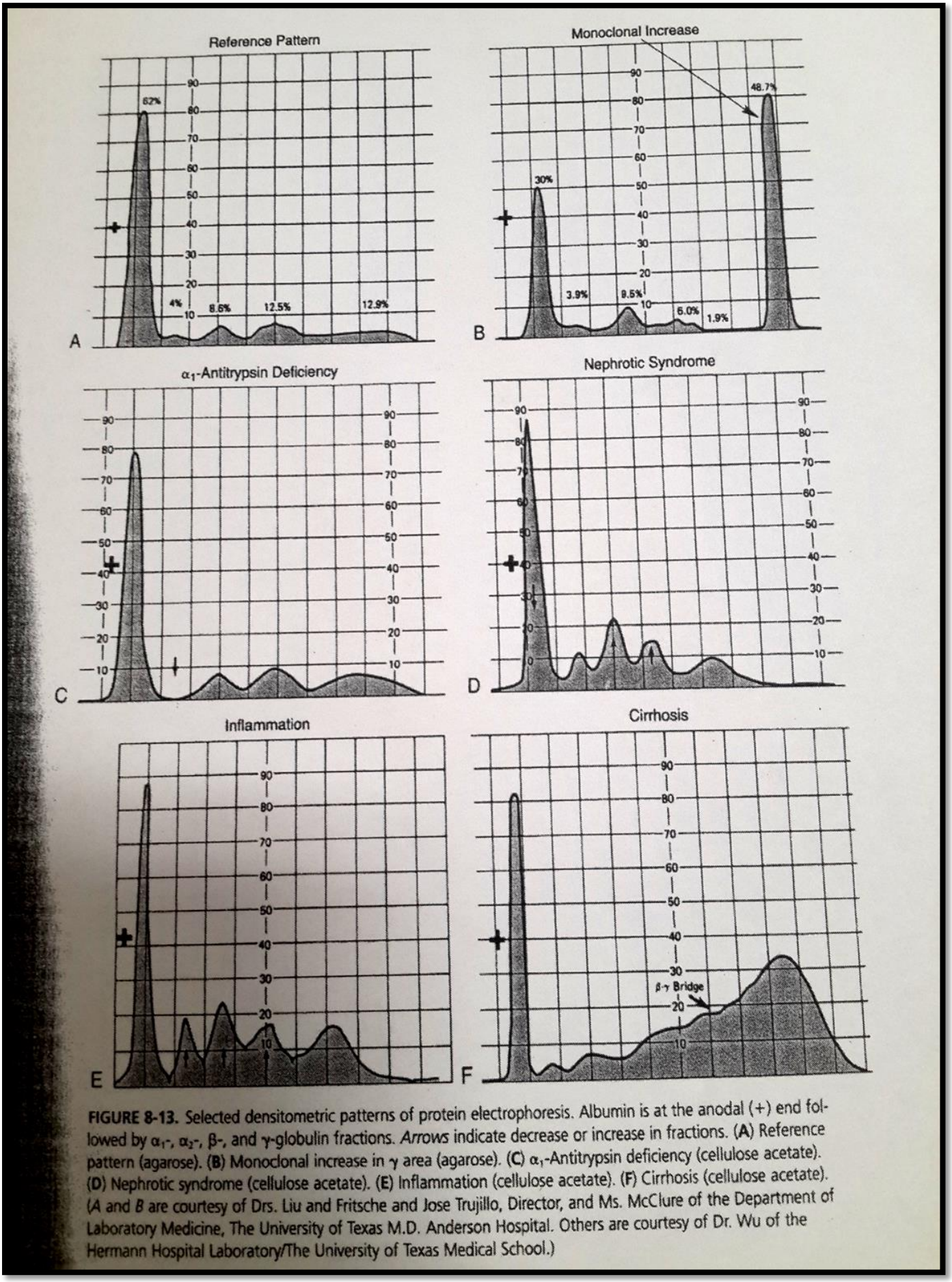


FIGURE 8-13. Selected densitometric patterns of protein electrophoresis. Albumin is at the anodal (+) end followed by α_1-, α_2-, β-, and γ-globulin fractions. *Arrows* indicate decrease or increase in fractions. (**A**) Reference pattern (agarose). (**B**) Monoclonal increase in γ area (agarose). (**C**) α_1-Antitrypsin deficiency (cellulose acetate). (**D**) Nephrotic syndrome (cellulose acetate). (**E**) Inflammation (cellulose acetate). (**F**) Cirrhosis (cellulose acetate). (*A* and *B* are courtesy of Drs. Liu and Fritsche and Jose Trujillo, Director, and Ms. McClure of the Department of Laboratory Medicine, The University of Texas M.D. Anderson Hospital. Others are courtesy of Dr. Wu of the Hermann Hospital Laboratory/The University of Texas Medical School.)