MIND MAPS IN CLINICAL CHEMISTRY

PART 2

Simmi Kharb

Bentham Books

Mind Maps in Clinical Chemistry

(Part II)

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PREFACE

This book treats clinical chemistry as an interdisciplinary subject to teach the importance of lab both for students in basic science as well as clinical medicine.

The approach to clinical chemistry in real clinical situations requires knowledge, experience and an integrated and clinically relevant model. I have attempted to bring this integrated model together in the book.

The book has been written in a lucid manner and is packed with practical approach of lab tests targeting undergraduate and also 1st postgraduate students of various streams of medicine. The reader should gain the knowledge and understanding of the value, limitations and interpretation of lab tests in modern medicine.

ACKNOWLEDGMENT & CONFLICT OF INTEREST

No potential conflict of interest is declared by the author. It is also declared the complete work is an individual effort by the author and there was no financial/ administrative/academic support availed from any individual/institution/ organization.

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Section I

Role of Biochemistry in Tumor Detection: Tumor Markers

CHAPTER 1

Role of Biochemistry in Tumor Detection

Learning objectives:

1. Enlist Specific Tumor markers for particular cancer.
2. Illustrate different oncogenes and their products.
3. Describe in detail Ectopic production of hormones.

Tumor Markers

Definition

A biochemical substance that may suggest presence of a type of cancer.
Tumor markers include substances, *e.g.* hormones, enzymes, other proteins and smaller peptides that are found/secreted in cells, tissues or body fluids (Table 1.1-1.3).

Uses of Tumor Markers

1. Screening (S)
2. Diagnosis (D)
3. Staging
4. Prognosis (P)
5. Monitoring treatment (M) (Follow-up)
6. Detection of recurrence or relapse (F).

Methods of Detection

- Chemical
- Immunological
- Molecular biological.

Methods of Evaluation

(To evaluate usefulness of tumor marker)

- Reference value
- Predictive value

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- Distribution of marker values
- Disease management value.

Table 1.1: Ideal tumor marker

	Easy and inexpensive to measure Levels should be stable	
	Class of Biochemical	*Example*
1.	Endogenous biochemical (increased production)	Hormone Enzyme Polyamine Oncofetal protein (CEA, AFP, hCG)
2.	Product of previously quiescent gene	Enzyme (PSA, antichymotrypsin) Cell surface antigen (β microglobulin)
3.	Receptors	Estriol receptor (breast CA) Androgen receptor (prostate CA)

Table 1.2: Biochemical classes used as tumor marker

	Class of Biochemical	*Example*
1.	Endogenous biochemical (increased production)	Hormone Enzyme Polyamine Oncofetal protein (CEA, AFP, hCG)
2.	Product of previously quiescent gene	Enzyme (PSA, antichymotrypsin) Cell surface antigen (β microglobulin)
3.	Receptors	Estriol receptor (breast CA) Androgen receptor (prostate CA)

Table 1.3: Specific tumor markers

Marker Substance (usefulness)		*Method of detection*	*Examples*	*Type of cancer*
1.	*Enzymes* (D,M,P)	Immunoassay, RIA	ALP CK LDH Neuron specific enolase ACP	Bone, liver, leukemia lung, breast, colon, prostate, ovary Liver, leukemia, lymphoma lung, carcinoid, melanoma, pheochromocytoma, pancreatic prostate CA, multiple myeloma, osteogenic sarcoma, bone metastasis
2.	*Hormones* (S,D,M,F) (S,D,P,M,F)	Immunoassay, RIA	PSA ACTH ADH Calcitonin Gastrin GH hCG HPL	prostate CA Cushing's, lung CA Lung, adrenal cortex CA medullary CA thyroid Glucagonoma Pituitary adenoma, renal, lung CA trophoblastic disease, testicular tumor Gonad, lung, breast CA

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			PTH	Liver, renal, breast, lung CA
			PRL	pituitary adenoma, renal, lung CA
			VIP	pancreas, bronchogenic CA, pheo-chromocytoma
3.	*Oncofetal antigens immunoassay*			
	(S,D,M,F)		AFP	Hepatocellular, germ cell CA
	(M,F)		CEA	colorectal, gastrointestinal, pancreatic, lung, breast cancers
4.	*Carbohydrate markers*:			
	mucin type	S,P,M Immunoassay	CA 125	Ovarian, endometrial CA
		M	CA 15-3	breast, ovarian CA
		F	CA 549	breast, ovarian CA
		F	CA 27.29	breast CA
			MCA	breast, ovarian CA
			DU-PAN-2	Ovarian, pancreatic, GIT, lung
			CA 19-9	Pancreatic GIT, hepatic CA
	Blood group antigens	Immunoassay (M,F)	CA 19-5	Ovarian, breast, GIT, colon CA
			CA 50	GIT, pancreatic CA
			CA 72-4	Multiple myeloma, CLL,
			CA 242	
5.	Proteins	Immunoassay (D,M,F) Immuno-assay	β_2 microglobulin	β-Cell lymphoma
			C-peptide	insulinoma
			ferritin	Liver, lung, breast, cancers
			Ig	leukemia
				Multiple myeloma, lymphoma
6.	*Receptors and other markers*	(M,P) Immunoassay (M,P)	Estrogen receptors	Breast CA
			Progesterone ®	
			Catecholamine metabolites	Neuroblastoma, pheochromo-cytoma,
			Urine metanephrine	
			VMA,HVA	
			Hydroxyproline	
			Polyamine	GIT, lung, rheumatoid CA
			Lipid associated	various cancers
			Sialic acid	
				Brain cancer
7.	*Genetic markers*	Immunohistochemistry		
a.	oncogenes		ras gene	AML, neuroblastoma
		(S,D,P)	C-myc gene	Leukemia, lymphoma
		(M,P)	C-erb β-2 & HER-2/ new gene	breast, ovarian, GIT CA
	Tumor suppressor gene	(D,M)	bcl-2 gene	Leukemia, lymphoma
		(S,D)	RB gene	retinoblastoma
		(S,D,P)	p53	Colon, liver, breast, lung CA
		–	p21	–
		(S,D)	APC gene	colorectal CA
		(S,D)	BRCA-1 & BRCA-2	Breast, ovarian cancers

S - Screening *M - Monitoring treatment*
D - Diagnosis *P - Prognosis* *F - Follow-up*

Section II
Special Topics

CHAPTER 2

Role of Clinical Laboratory

Learning objectives:

1. Discuss Outline of Lab work flow.
2. Describe setting of a Clinical Laboratory.

A clinical chemistry laboratory performs a large number of biochemical analytes on body fluids which can give answers to specific clinical questions about an individual patient. Such analyses are usually requested in blood and urine samples to aid in diagnosis or treatment.

The entire process from a request for analysis to receipt of a result involves many steps and can take several hours. Also, quality assurances for analyte are performed to ensure that produced results are analytically and clinically valid.

The purpose and function of laboratorians are to assist clinicans in:

1. Confirming or rejecting a diagnosis
2. Providing guidelines in patient management
3. Establishing a prognosis
4. Detecting or screening disease
5. Monitoring follow-up

Outline of Lab Work Flow (**Fig. 2.1**)*:*

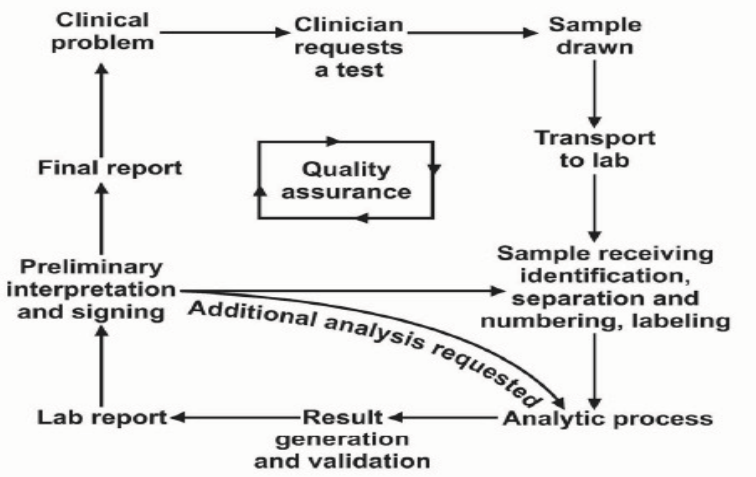

Fig. 2.1: Steps involved in generation of results in clinical lab

Setting up a Clinical Laboratory

A cost-effective and successful laboratory service is one that results from careful planning and design to meet current and future needs of personnel, equipment and space.

Laboratory organization:

Key elements:

Workplace
Staff
Tasks

Steps in laboratory design process:

1. *Preparation* : to assess: needs
   staff requirement
   technology
   identify space for workplace in terms of:
   office: lab staff
   medical staff
   personal facilities:
   conference/library area

2. *Function* : determine current and future needs of laboratory and provision for upgradation in terms of: activities
   flow of people
   equipment

3. *Schematic design:*
   S*tructural design*: Develop and use a project schedule, consider the following points:
   i. architect design
   ii. cost
   iii. *system options*: plumbing, electricity, ventilation, air-conditioning
   iv. *lab safety measures:* fumehood, biological safety cabinets should be planned away from door ways
   v. *space requirements:* 150 to 1200 square feet for open lab concept, 27 to 40 square feet per hospital bed (lab counter: 2–3 feet wide).

4. *Design*: Interiors: Lab fixtures/modulator furniture
   Base cabinets
   Noise control

5. *Construction*: Actual construction processes should be according to regulatory and safety compliance codes. (see Fig. 2.2)

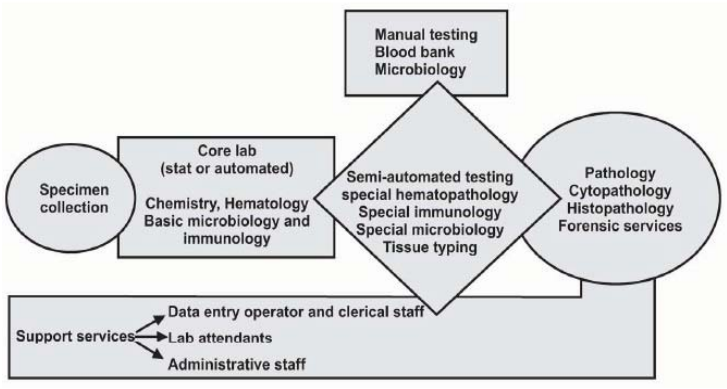

Fig. 2.2: Hospital laboratory flow diagram

Medicolegal Concerns

1. *Consent* - processing informed consent from the patient
2. *Confidentiality* - patients are entitled to strict confidentiality concerning their laboratory measurements and results. Release of any patient information to non-health care entities (*e.g.* lawyer, insurance companies, friends, *etc.*) must be authorized by the patient especially in case of HIV positive blood examination.

Financial management in case of payment cases and reimbursement is important.

Accreditation. All lab performing moderate or highly complex testing must participate in accredited proficiency program and be tested in every procedure for which the lab is certified.

Lab Accreditation

Accreditation is formal recognition, authorization and registration of a laboratory having demonstrated its capability, competence and credibility to carry out the tasks it is claiming to be able to do. Also, the laboratories get feedback regarding their performance in accordance with international criteria for technical competency. Lab accreditation is a means for improving customer and clinician confidence in test reports and the acceptance of reports with confidence.

NABL Accreditation (National Accreditation Board for Testing and Colibration Laboratories).

NABL is an autonomous body under the aegis of Department of Science and Technology (DST), Government of India that provides accreditation to various types of laboratories. NABL is signatory of

CHAPTER 3

Recent Advances in Clinical Lab

Learning objectives:

1. Describe Biosensors and Biodetectors.
2. Explain role of Molecular probes in Lab diagnosis.
3. Discuss Nanotechnology.

Techniques

Mass Spectrometry and MALDI-TOF MS

Mass spectrometry is used to measure molecular weight of proteins, to detect postranslational modification of proteins and peptides and in proteomic research.

Matrix Assisted Laser Desorption Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF-MS) is a sensitive and powerful technique for the characterization of proteins, peptides, and various biomolecules.

After irradiation by a short laser pulse, protein in sample gets ionized and desorbed from matrix in which it was dispersed. On exposure to voltage gradient in vacuum, ionized protein accelerates at a rate proportional to its mass to charge ratio. After a time (TOF) each molecule reaches the detector depending on its molecular mass.

Proteomics

Proteome

A proteome is defined as full complement of proteins produced by a particular genome.

Proteomics

Proteomics is field of study of protein properties. It is defined as qualitative and quantitative comparison of proteomes with the goal of further unraveling biological process.

It involves extraction of protein by 2D SDS - PAGE, extraction of individual protein spots, followed by digestion with proteases. Small peptides thus obtained are sequenced by mass spectrometry and thus protein is identified.

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High Performance Liquid Chromatography (HPLC)

A highly sensitive, specific and powerful chromatographic technique for high resoution separation and purification of proteins, peptides and amino acids.

Protein mixture is applied to narrow columns packed with noncompressible matrix of beads coated with a thin layer of stationary phase. Components are then eluted by chromatography (isocratic or gradient) and monitored by ultraviolet absorption or fluorescence.

ISE (Ion-selective electrode)

ISE:

ISE is a type of potentiometric electrode consisting of a membrane selectively permeable to single ionic species (Table 3.1).

Table 3.1: ISE

	Types	*Membrane*	*Examples*
1.	Glass electrode	Glass	H^+ selective (pH), Na^+, F^-, Cl^-
2.	Solid state electrode	Crystalline inert matrix	
3.	Liquid ion-exchange electrode	Inert solvent in a matrix of PVC (in which ion-selective carrier substances are dissolved)	K^+, NH_4^+, Ca^{2+}, H^+
4.	Gas electrode (special application of pH glass electrode)	gas permeable membrane, *e.g.* Teflon	CO_2, NH_3

Applications: ISE

ISE are easy to use, economically easy to transport, ISEs are rapid in use, respond over a wide range of concentrations and do not destroy the sample.

Biosensors

A biosensor is a sensing device made up of a combination of a specifical biological element and a transducer which can convert as a biological or biochemical signal or response into a quantifiable electrical signal (Fig 3.1, 3.2; Table 3.2).

Analyte → Bioelement → Sensor → Electrical signal

Types of Biosensor

Bioelement	*Sensor element*
Antibody	Electric current
Enzyme	Electric potential
Microbial	EM radiation
Nucleic acid	Mass
Polysaccharide	Temperature
Tissue	Viscosity

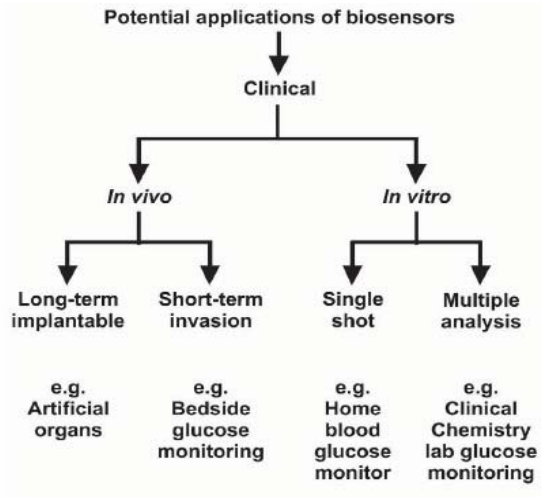

Fig. 3.1: Potential applications of biosensors

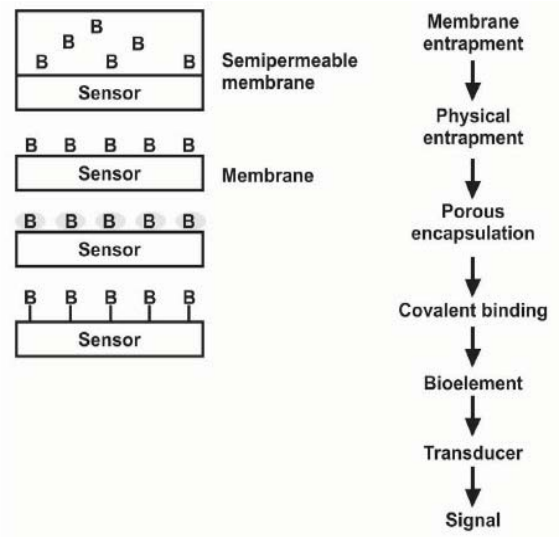

Fig. 3.2: Biosensor

Table 3.2: Types of Biosensor

	Type	*Bioelement*	*Sensor*
1.	Resonant biosensor	Antibody	When antigen (analyte) gets attached to membrane and there occurs change in membrane mass. The resulting change in resonant frequency is measured.

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CHAPTER 4

Automation in Clinical Laboratory

Learning objectives:

1. Discuss steps for a complete analysis or unit operation.
2. Illustrate systems available for automation.

Process where an analytical instrument performs analytical tests with only minimal involvement of an analyst is *automation*.

Steps in for a Complete Analysis or Unit Operation

1. Specimen identification
2. Specimen preparation
3. Specimen delivery
4. Specimen handling and transport
5. Specimen processing
6. Sample transport and delivery
7. Reagent handling and storage
8. Reagent delivery
9. Chemical reaction phase
10. Measurement approaches
11. Report processing, data handling, quality control and reporting

Systems Available for Automation

1. *Centrifugal analyzers:* Use discrete pipetting to load samples and reagents sequentially into discrete vessels in a rotor and samples are subsequently analyzed in parallel.
2. *Benchtop analyzer or batch analyzer:* Specimens are presented sequentially on batch basis and appropriate tests can be selected for each specimen.
3. *Continuous flow analyzers:* Single-channel continuous flow batch analyzer that can provide multiple test results on the same specimen.
4. *Random access analyzer:* A multi-channel analyzer where tests performed are selectable through use of differentials or packs of reagents.
5. *Urine analyzers:* Similar to serum analyzers, but, require low limit of detection and expanded linearity because of broad range of concentration of many urine constituents.

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6. *Workstations:* Workstations are clinical laboratory work station devoted to defined task, *e.g.* chemistry profile, complete blood count, hormone testing, PCR, urinalysis containing appropriate laboratory instrumentation to carry out the task is available.
7. *Remote automated laboratory system (RALS)*: RALS uses a robot to introduce patient sample and analyzes the sample and sends the result to central monitoring workstation by a network interface. There the results are viewed, accepted or rejected by trained medical technologist before being released for clinical use. Usually RALS approach allows testing to be carried at point-of-care testing (POCT), while maintaining the central laboratory control over the process.

Thus, the significant improvement in quality and time required for reporting of lab tests occurs with combination of well-designed automated instrumentation with good analytical methods and effective quality assurance programs.

Although the cost of automation may initially seem to be high, substantial cost savings usually accrue by a reduction in the number of staff needed to process the workload.

Questions:

1. What are the systems available for automation?
2. What is the significance of automation in a clinical setup?

Bibliography:

Burtis, C., Ashwood, E., Bruns, D. and Tietz, N., 2008. Tietz Fundamentals Of Clinical Chemistry. St. Louis, Mo.: Saunders Elsevier.

Keith Wilson, John M Walker. Principles and techniques of biochemistry and molecular biology. 7th edition. Cambridge: Cambridge University Press; 2017.

CHAPTER 5

Clinical Laboratory Information System

Learning objectives:

1. Illustrate Lab Information system.
2. Explain the importance of Telemedicine.

LIS integrates clinical laboratory with medical staff and most departments of a health care organization (Fig. **5.1**).

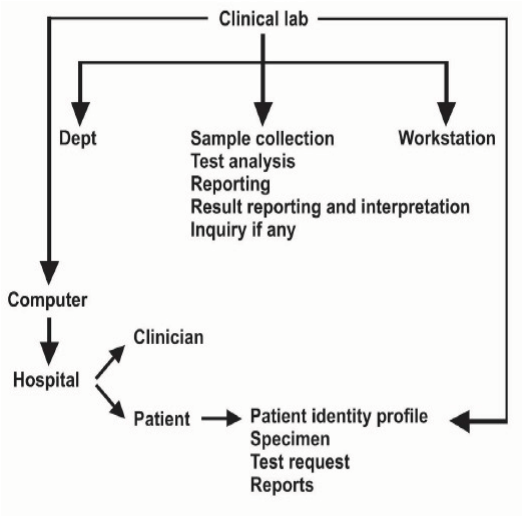

Fig. 5.1: Lab Information System (LIS)

Telemedicine

The use of telecommunications as part of telemedicine ranges from use of digital network image and text transfer through use of parallel computers and cluster of high-performance workstations.

These systems link hospitals, clinics, extended care facilities, physician offices, offering real-time communication and video conferencing to develop networked regional health care facilities.

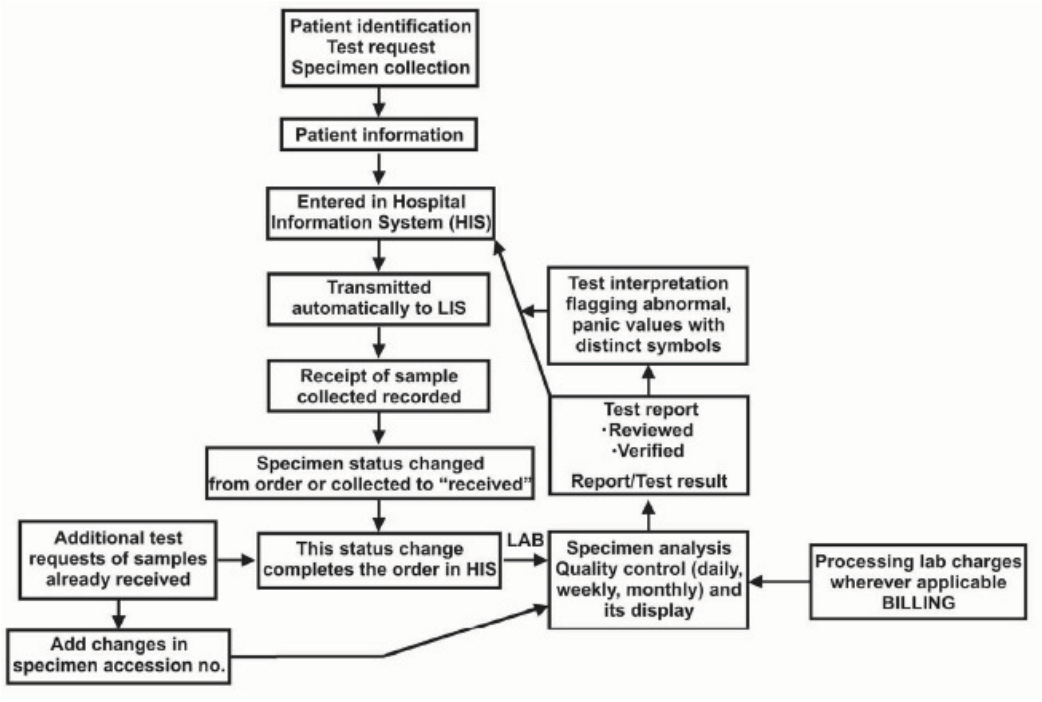

Fig. 5.2: Medical informatives

Questions:

1. Describe Lab Information System (LIS).
2. What are the uses of Telemedicine?

Bibliography:

Burtis, C., Ashwood, E., Bruns, D. and Tietz, N., 2008. Tietz Fundamentals Of Clinical Chemistry. St. Louis, Mo.: Saunders Elsevier.

Keith Wilson, John M Walker. Principles and techniques of biochemistry and molecular biology. 7th edition. Cambridge: Cambridge University Press; 2017.

CHAPTER 6

Role of Clinical Laboratory in Organ Transplantation Program

Learning objectives:

1. Enlist clinical tests for diagnosis before organ transplantation.
2. Describe Organ Specific Monitoring.

Clinical Tests for Diagnosis (Table 6.1)

1. *Routine:*
   - Routine clinical chemistry
   - Coagulation
   - Microbiology
2. *TDM:*
   - For measuring and optimizing individual dosage of immunosuppressive drugs and toxic drugs.
3. *Indicators of graft rejection:*
   - Biochemical
   - Serological
   - Molecular markers of onset of an infection or rejection in donor and recipient
4. *Preparing for transplant:*
   - Tissue typing
   - ABO matching
5. *Interaction between lab and clinical staff (for consulting various issues)*

   Since transplant medicine is a rapidly developing branch of clinical medicine, in future, clinical labs will have to provide analytical, biochemical, pharmacological, epidemiological services to monitor recipients of such grafts.

Table 6.1: Organ specific monitoring

Organ	*Tests*
Kidney	S. creatinine GFR

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	Urine:
	protein (β$_2$ microglobulin, α$_2$ macroglobulin)
	Tubular enzymes
	alanine aminopeptidase
	γ glutamyltranspeptidase
	Lysozyme
Liver	S. bilirubin
	S. bile acids
	ALP
	ALT
	GDH
	GGT
	Coagulation tests:
	Prothrombin time
	Urine analysis
	Plasma pancreatic amylase
Pancreas	Trypsin inhibitor
Heart	Plasma phospholipase A$_2$
	CK-2
	CK-2/CK-1
Lung	Bronchoalveolar lavage fluid (BAL):
	↑ lymphocytes, monocytes
	↑ cytokine
	↑ IL-4 gene
	↑ CD^{8+} cells
	Blood gases

Questions:

1. What are the clinical tests used in diagnosis of organ transplantation?
2. Elaborate the tests done for monitoring of different organ transplantation?

Bibliography:

Burtis, C., Ashwood, E., Bruns, D. and Tietz, N., 2008. Tietz Fundamentals Of Clinical Chemistry. St. Louis, Mo.: Saunders Elsevier.

Keith Wilson, John M Walker. Principles and techniques of biochemistry and molecular biology. 7th edition. Cambridge: Cambridge University Press; 2017.

Miller, 1., 2020. Handbook Of Specimen Collection And Handling In Microbiology. [online] Stacks.cdc.gov. Available at: <https://stacks.cdc.gov/view/cdc/7590>.

Murray, R., Bender, D., Botham, K., Kennelly, P., Rodwell, V. and Weil, P., 2012. Harpers Illustrated Biochemistry 29Th Edition. Blacklick: McGraw-Hill Publishing

Tietz, N., 1987. Fundamentals Of Clinical Chemistry. Philadelphia: W.B. Saunders.

CHAPTER 7

Setting of a Clinical Laboratory

Learning objectives:

1. Discuss steps in Laboratory design process.
2. Illustrate Hospital laboratory flow diagram.

An economical and advantageous laboratory facility is defined from the outcomes of meticulous designing and planning, to answer the needs in terms of machinery/ equipments, staff and space requirement-availability for the present as well as in future.

Steps in laboratory design process:

1. Preparation steps
    a. Assessment of
        i. Requirements/ requests
        ii. Personnel required
        iii. Techniques and expertise
    b. Recognize space/ area required for workplace especially for facilities needed for the personnel and office work:
        i. Laboratory personnel
        ii. Physicians
        iii. Conference, meeting and library area
2. Function – Define the present as well as future requirements of laboratory and possibility for upgradation of
    a. Procedures and techniques
    b. Manpower workflow
    c. Machines and equipments
3. Schematic design: Basic operational design: prepare and utilise a project scheme:
    a. Structural design from architect/ engineer
    b. Capital involved/ required
    c. System operations: establishing electrical facility, air ventilation and conditioning
    d. Safety cabinets like fumes hood/ laminar hoods and biological safety cabinets; they have to be established away from doorways
    e. Requirements for area and space: 150– 1200 square feet for the concept of open laboratories, 2-3 feet wide laboratory counters, 27– 40 square feet area for each hospital bed, *etc.*

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4. Design of Interiors

• Modulator Furniture and Laboratory fixtures
• Cabinets/ racks at the base
• Modulation and control of Noise/ sound

5. Construction: Definite physical processes of construction in accordance with regulatory and conformity of safety rules/ codes.

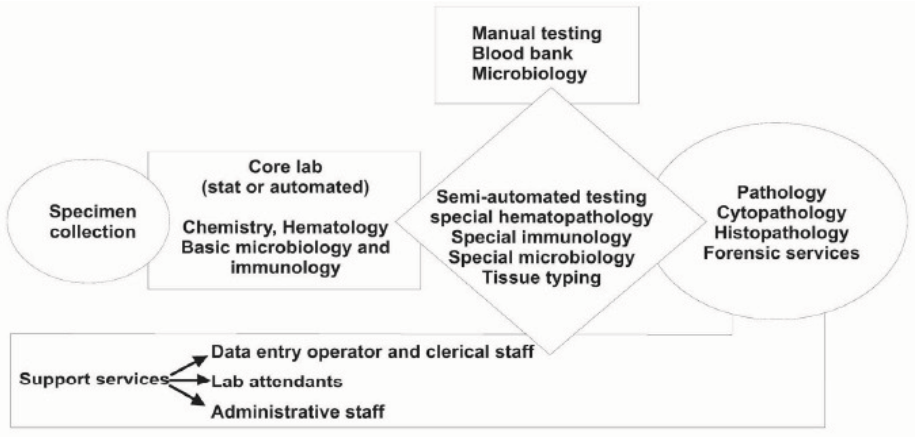

Fig. 7.1: Flow Diagram of a Hospital Laboratory

Medicolegal Concerns

1. Patient's consent – dealing out informed patient's consent
2. Patient – physician confidentiality – every individual is eligible for firm terms of confidentiality regarding their lab tests and results. Release or Publication of any information of the patient to non-health care units (like friends, insurance companies, lawyers, *etc.* need to be sanctioned by the individual/ patient especially in cases involving examination of HIV+ve blood.

Management of finances in terms of cases where payment or reimbursement is done are important.

Accreditation

All laboratories carrying out moderate or extremely composite testing need to compulsorily partake in accredited/ certified expertise program and be verified in every technique for which the laboratory is certified.

Questions:
1. Discuss the steps in laboratory design process?
2. Describe flow diagram of a hospital laboratory.
3. What are medicolegal concerns?

Bibliography:

2011. Prudent Practices In The Laboratory. Washington, D.C.: National Academies Press.
Belloc, H., 1967. On. Freeport, N.Y.: Books for Libraries Press.
Burtis, C., Ashwood, E., Bruns, D. and Tietz, N., 2008. Tietz Fundamentals Of Clinical Chemistry. St. Louis, Mo.: Saunders Elsevier.
Keith Wilson, John M Walker. Principles and techniques of biochemistry and molecular biology. 7th edition. Cambridge: Cambridge University Press; 2017.
Luxon, S., 1992. Hazards In The Chemical Laboratory. Cambridge: Royal Society of Chemistry.
Miller, 1., 2020. Handbook Of Specimen Collection And Handling In Microbiology. [online] Stacks.cdc.gov. Available at: <https://stacks.cdc.gov/view/cdc/7590>.
Murray, R., Bender, D., Botham, K., Kennelly, P., Rodwell, V. and Weil, P., 2012. Harpers Illustrated Biochemistry 29Th Edition. Blacklick: McGraw-Hill Publishing
Tietz, N., 1987. Fundamentals Of Clinical Chemistry. Philadelphia: W.B. Saunders.

CHAPTER 8

Various Body Fluids

Learning objectives:

1. Describe Cerebrospinal fluid.
2. Discuss Synovial fluid.
3. Explain different exudates in body.

Various body fluids include (Tables **8.1** and **8.2**):

- Cerebrospinal
- Synovial
- Serous body fluids:
  - Pleural
  - Pericardial
  - Peritoneal

Cerebrospinal Fluid (CSF)

Table 8.1: Cerebrospinal fluid (CSF)

Features:		
Produced @ 500 mL/d		
70% derived from ultrafiltration and secretion through choroidal plexus		
Remainder from ependymal lining of ventricles and cerebral subarachnoid space		
Total CSF volume:		
	Adults	90–150 mL
	Neonates	10–60 mL

Blood Brain Barrier (BBB)

It maintains relative homeostasis of CNS environment during acute perturbations of plasma components. Substances tightly regulated by transport system in BBB are:

- H^+
- K^{2+}
- Ca
- Mg
- HCO^3

Freely diffusible substances via BBB:
- Glucose
- Urea
- Creatinine

Substances passively diffused across BBB:
- Proteins

Table 8.2: Lab tests on CSF

CSF pressure		
Total and differential cell count		
Glucose (CSF/plasma ratio)		
Proteins (CSF/plasma ratio)		
Cultures	:	bacteria, fungi, viruses, Myco. tuberculosis
Stains	:	gram's stain, acid-fast stain
Antigens	:	bacteria, fungi
PCR	:	TB, viruses
Cytology		
Electrophoresis	:	proteins
VDRL	:	syphilis

Diseases Detected by CSF Examination (Table 8.3)

Meningitis (bacterial, fungal)
Intracranial hemorrhage:
- Subarachnoid
- Subdural

Central syphilis
Malignancy

Table 8.3: Chemical analysis of CSF

Chemical analysis: CSF	*Conditions*
Total protein (high):	
	Infections (meningitis)
	Hemorrhage
	Autoimmune disease
	Myopathies
	Uremia
	Drugs:
	ethanol
	phenytoin
	Meningitis
	Malignancies
α macroglobulin	Inflammation
β_2^2 microglobulin	

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CRP		Meningitis (bacterial)
Glucose (low)		Meningitis (bacterial, malignant, hemorrhage)
Lactate	<25 mg/dL	Viral meningitis
	>35 mg/dL	Bacterial meningitis
LDH	High	Bacterial meningitis
CK - BB	High	Demyelination disease: Seizure Stroke Tumor Meningitis
Ammonia	High	Hepatic encephalopathy
Glutamate	High	Hepatic encephalopathy

Synovial Fluid

Salient features
Imperfect ultrafiltrate of plasma combined with hyaluronic acid derived from synovial cells Acts as a lubricant and adhesive
Provides nutrition to avascular articular cartilage Small ions, *e.g.* glucose, urea freely cross into joint (Table **8.4**)

Table 8.4: Tests for synovial fluid

Leukocyte count	:	Total and differential
Stain	:	Gram's, fungal, acid-fast
Culture	:	Aerobic and anaerobic bacteria
Crystal examination (microscopy)	:	Monosodium urate, calcium pyrophosphate, calcium oxalate, lipid Basic calcium phosphate
PCR	:	Bacterial, mycobacterial DNA
Biochemistry	:	Glucose Protein Lactate Complement RhF ANA Enzymes: LDH Acid phosphatase

Pleural Fluid (Tables 8.5 and 8.6)

Salient Features

Plasma filtrate derived from capillaries of parietal pleura.
Present in pleural cavity in small amounts.
Accumulation of fluid is called *Effusion*.

CHAPTER 9

Organ Panels

Learning objectives:

1. Explain Organ panels and related clinical features.

1. *Anemia*
   - Complete hemogram
   - Retics
   - PBF:
     - Microcytic - Iron panel, ESR
     - Macrocytic - Vitamin panel - B_{12} folate, Thyroid - TSH
2. *Acid-base balance*
   - pH
   - pCO_2
   - pO_2
   - HCO_3
   - O_2 saturation
   - O content
   - Base excess
3. *Bone/joints/arthritis*
   - ESR, Uric acid, Albumin, Total protein, Ca, P, ALP, UA, Osteocalcin
   - CRP
   - RhF
   - ANA
4. *Cardiac*
   - CK - MB
   - Myoglobin
   - Troponin - I
   - BNP

5. *Coma*

Metabolic panel Plus calcium	Toxicology Salicylate Ammonia Anion gap		Acid-base organic acids: Alcohol Lactic acid Osmolality

6. *Diabetes*

Metabolic panel	HbA_{IC}	Anion gap	Lipid profile

7. *Nutritional supplementation/support*

Basic metabolic panel	Others: Mg Albumin Ca, P ALP TG

8. *Routine health check up*
   - Complete blood count
   - Metabolic panel
   - Lipid panel
   - UA
   - GGT
   - TSH
9. *Hemolysis*
   - CBC
   - Bilirubin
   - Haptoglobin
   - Serum and Urine - Free Hb
   - Reticulocyte count
10. *Hypertension*
    - Metabolic panel
    - U. free cortisol
    - Renin

    Thyroid panel

    U. metanephrines
11. *Iron panel*
    - S. iron
    - TIBC
    - % Saturation
    - Ferritin
12. *Lipid panel*
    - TG
    - Cholesterol : total, HDL (direct), LDL, VLDL
    - *Special*:
      - Apolipoproteins
      - Lipoprotein (a)

13. *Liver function:*
    Albumin Bilirubin SGOT/PT GGT
    Prothrombin time ALP
14. *Neonatal screening for:*
    PKU (Phen)
    BCAA urea (Leu)
    Galactosemia (Gal -I-P- Uridyl transferase)
    Homocysteine (Methionine)
    Hypothyroidism (Thyroxine, TSH)
    Sickle cell (Hb electrophoresis)
15. *Pancreas*
    Amylase
    Lipase Calcium TG Glucose
16. *Kidney*
    Metabolic panel Mg, Ca/P CBC
    Albumin
    U. protein 24 hrs
    Creatinine clearance.
17. *Thyroid*
    T3, T4, TSH
18. *Toxicology*
    Amphetamines
    Benozdiazepines
    Barbiturates
    Cocaine metabolites
    Ethanol (alcohol)
    Opiate metabolites
    Phencyclidine
    Methadone.

Questions:

1. Write the names of tests done for the following :
   a) Anemia
   b) Cardiac Diseases
   c) Diabetes
   d) Hypertension
   e) Thyroid Disorder

Section III
General Lab Techniques and Instruments

CHAPTER 10

General Laboratory Techniques and Procedures: Introduction

Learning objectives:
1. Enumerate different instruments and equipments in Clinical chemistry lab.

Instruments

A clinical chemistry lab requires the following lab supplies:

1. *Chemicals and related substances:*
   - Reagent grade chemicals:
     - Laboratory grade LR
     - Analytical grade AR
     - HPLC grade or ultrapure
2. Reagent grade water:
   - Distilled water
   - Deionized water
   - Reverse osmosis
3. *Laboratory supplies:*
   - i. Glassware: Tubes, flasks, cylinders, funnels
     Thermal resistant, borosilicate glassware: Pyrex, corning
   - ii. Plasticware
     - i. Polyethylene and polypropylene: used in disposable plastic-ware
     - ii. Polycarbonate: used in centrifuge tubes
     - iii. Fluorocarbon resin (Teflon): inert, high corrosion resistance at extreme temperatures
     - iv. Synthetic and rubber tubing: Tygon
4. Volumetric equipments:
   - Pipettes
   - Dispensers
   - Burette
   - Flasks
5. Centrifuges
6. Mixers and homogenizers
7. Balance and weighing instruments

8. Thermometers
9. Analytical techniques and instrumentation
    i. Colorimeter
    ii. Spectrophotometer
    iii. Spectrophotofluorimeter
    iv. Flame emission spectrophotometer
    v. Atomic absorption spectrophotometer
    vi. Nephelometer and turbidimeter
    vii. Scintillation (counting detectors)
    viii. Potentiometry
        Electrodes
            inert metal: Hydrogen
            ion - selective
            ion - exchange
            gas electrode: pCO_2
        Biosensors
    ix. Electrophoresis
    x. Chromatography/Mass spectrometry

Questions:

1. What are the instruments required in a clinical chemistry laboratory?
2. Write the names of different analytical techniques.

Bibliography:

2004. Laboratory Biosafety Manual. Geneva: World Health Organization.
2011. Prudent Practices In The Laboratory. Washington, D.C.: National Academies Press.
Burtis, C., Ashwood, E., Bruns, D. and Tietz, N., 2008. Tietz Fundamentals Of Clinical Chemistry. St. Louis, Mo.: Saunders Elsevier.
Kaplan, L. and Pesce, A., 2010. Clinical Chemistry. St. Louis, Mo.: Mosby/Elsevier.
Keith Wilson, John M Walker. Principles and techniques of biochemistry and molecular biology. 7th edition. Cambridge: Cambridge University Press; 2017.
Luxon, S., 1992. Hazards In The Chemical Laboratory. Cambridge: Royal Society of Chemistry.
Miller, 1., 2020. Handbook Of Specimen Collection And Handling In Microbiology. [online] Stacks.cdc.gov. Available at: <https://stacks.cdc.gov/view/cdc/7590>.

CHAPTER 11

General Lab Techniques

Learning objectives:

1. Describe Paper electrophoresis.
2. Discuss about Chromatography and ELISA.
3. Enumerate applications of Radio-isotopes.
4. Explain Mass Spectroscopy, its working, types and applications.

Paper electrophoresis

Separation of different fractions of serum proteins by paper electrophoresis

Electrophoresis is a method of separation of different substances based on their different rates of migration in an electric field.

The rate of migration depends upon:

- size
- change on the particles
- strength of electric field

When the latter (electric field) is held constant, different charged particles, which might be initially at one position in a solution will move at different rates, and after a different time period they will be found at different distances from the starting point. When a voltage is applied to two electrodes in a conducting solution, an electrical potential is set up between them. Charged particles in the solution will move under the influence of electric field, the negative particles moving towards the positive electrodes and the positive particles moving towards the negative electrode.

Theory

Support Media

For example, Whatman filter paper no. I, III acts as a support media in paper electrophoresis. It will absorb a certain amount of buffer solution that is needed to carry the electric current.

Other media: Cellulose acetate
agar
agrose gel
polyacrylamide gel

Sample Application

Mixture to be separated is added as a streak near one end of the medium, connections are made for an electric current. At the two ends, the potential is applied. The substance moves along the strip of supporting medium at different rates, depending upon their size and charge.

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After a suitable time, the current is stopped and strip is taken from the apparatus. The strip is then usually fixed and stained that will give color with the material being separated.

Quantitation

After the position of various components have been located by staining, they may be quantitated by several ways:

i. Estimated semiquantitatively by noting relative sizes and intensity of separate stained portions.
ii. Strip may be cut into sections and stained material in separated sections is eluted and amount is determined photometrically: scanning absorptionmeter.
iii. Different portions can be scanned by densitometer.

Significance

i. Used for separation of amino acids and proteins in diseases, *e.g.* nephrosis, multiple myeloma, rheumatoid arthritis, agammaglobulinemia.
ii. Plasma proteins, lipoproteins, isoenzymes can be separated.
iii. Urinary amino acids, VMA can be determined.

Mobility

It depends upon:

- charge
- size
- shape—spherical particles move faster than rod-shaped
- on composition of buffer: Rate of migration increases as pH of buffer is removed away from pI.

At pH 8.6, all proteins are anions and good separations can be obtained, and with decreased ionic strength, bands become diffuse.

Voltage, current and heating effect

i. Mobility of particles is proportional to potential gradient along the strip.
ii. Variation in design of tanks and ionic strength greatly modifies the potential drop between electrodes and ends of paper strips. This potential drop will also vary according to a number of strips in circuit.

Procedure

A 36 cm × 3 cm strip of Whatman filter paper is taken.
9 cm away from one end of strip, make a pencil mark line.
Apparatus: Consists of electrophoretic chamber having a bridge between electrodes, on the two edges of bridge on one side anode is present and other side cathode is present.
Barbitone buffer of pH 8.6 is poured into those chambers.

Barbitone Buffer

- 5 g Na barbitone
- 3.2 g Na acetate
- 4.4 ml 1N HCl
- make final volume to 1 L with distil water.

Soak filter paper with buffer, place the strip between 2 folds of filter paper to blot/soak excess buffer. Put it on bridge such that its ends dip into barbitone buffer and pencil line points towards cathode side.

Application of Sample

With micropipette apply sample in the form of streak on mark (10 to 15 µl) in such a way that it does not spread, place the lid over chamber and allow it to stand for 10 to 15 min.

Power Supply

A voltage of 3 V/cm of strip at constant current of 2 mA strip is applied, and power is run for 10 to 18 hours. Then, remove the strip, let it dry by incubating in oven at 100°C for 10 to 15 min to denature and fix the proteins and the process is termed as fixation.

Staining

It is carried out by light green dye—15 g dye, 20 ml alcohol, 5 ml glacial acetic and make final volume to 100 ml with distil water.

Washing and Destaining

Wash 2 to 3 times with 20% acetic acid excess green color is removed. Wash strip thoroughly with water and again dry it in oven or air.
Albumin moves most rapidly, band is obtained at greatest distance from start line.

Normal Values

Plasma proteins	6.3–7.9	g%
Albumin	3.7–5.3	g%
Globulin	1.8–3.6	g%
Fibrinogen	200–400	mg%
A/G ratio	2.5 :1 to 1.2:1	

Clinical Significance

- For qualitative assessment of change in relative amounts of globulin.
- For quantitation of bands obtained on electrophoresis (Fig 35.1).

1. Chronic liver disease	↓ albumin ↑ α_1, α_2 globulin
2. Infective hepatitis	↑ γ-globulin

Section IV

Practical Biochemistry Exercises

CHAPTER 12

Carbohydrates

Learning objectives:

1. Illustrate scheme for identification of unknown carbohydrates using different tests.
2. Enlist biologically important carbohydrates.
3. Discuss Osazone crystals.

Introduction

Carbohydrates are defined chemically as aldehydes or ketone derivatives of higher polyhydric alcohols.

Biomedical Importance

1. Source of energy
2. Serves as cell membrane component
3. Used for synthesis of nucleic acid and other biological compounds.

Classification

1. Sugars—monosaccharide
   Non-sugars—di, oligo and poly-saccharide
2. Aldose—For example, Glucose
   Ketose—For example, Fructose.

Monosaccharides

Those carbohydrates that cannot be hydrolyzed into simpler carbohydrates. They may triose, tetrose, pentose, hexose, heptose or octose depending on the number of carbon atoms they possess and occur as aldose or ketose depending upon aldehyde or ketone group present.

Reactions of Monosaccharides (Figs. 12.1 and 12.2)

Chemical properties depend on activity of aldehyde or ketone group:

1. Reducing properties: Benedict's
   Barfoed's

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2. Osazone formation
3. Furfural formation: Molisch, Seliwanoff's

Scheme

If an unknown carbohydrate solution is provided, the following scheme of tests is followed (Fig 36.2):

Molisch Test

Reagent: 5% naphthol in absolute alcohol
Procedure: To 2 cc unknown solution in a test tube, add 2 drops of α-naphthol, mix, pour 2 cc conc. H_2SO_4 along the sides of the tube and gently rotate between your palms.

Purple ring at the junction of the two liquids implies presence of carbohydrates.

Principle: Carbohydrates react with strong mineral acids to yield furfural or its derivatives, which in turn, condenses with organic phenols (*e.g.* α-naphthol) to give a violet colored complex.

Precautions

i. Use dry tubes
ii. H_2SO_4 should be fresh (otherwise test turns brown)
iii. α-naphthol should be fresh (otherwise test turns green)
iv. Pour the acid slowly along the side of the tube.

Iodine Test

Reagent: 0.05 N iodine solution
Method: Take 2 ml of unknown solution, add two drops of iodine solution. Appearance of blue color implies that polysaccharides are present.

Violet: amylodextrin
Red: erythrodextrin
Portwine (reddish brown): glycogen

Negative iodine test implies that it may be mono- or disaccharide.

Principle: On carbohydrate molecules, iodine gets adsorbed to form a cage-compound which is blue in color.

Benedict's Test

Reagent: $CuSO_4$ – gives cupric ions
Na_2CO_3: Weak alkali
Na Citrate: Stabilizing agent

Method: Take 5 cc of Benedict's reagent, add 8 to 10 drops or 0.5 ml of unknown solution. Boil it for 2 minutes. Allow it to stand and cool. See color of the deposits.

Color	g%	
Green	0.5	+
Yellow	1	++
Orange	1.5	+++
Red	2	++++

Principle: Under alkaline conditions, copper sulfate is reduced by reducing sugars:

$$CuSO_4 \rightarrow \underset{Cu}{CuO} + Na_2SO_4 \xrightarrow{\text{Reducing sugar}} {}_2O \text{ (colored)}$$

Saccharoid: Non-sugar reducing agents, *e.g.* $CHCl_3$, vitamin C, uric acid. They do not interfere with this test.

Clinical importance: In screening of DM.

Fehling's Test

Fehling's A: $CuSO_4$ – gives cupric ions
Fehling's B: Na K tartarate + KOH

Procedure: Take 2.5 cc each of A and B, mix, add 1 drop of urine and heat. Appearance of red color indicates positive test.

Fehling's is more sensitive and less specific than Benedict's test.

$CHCl_3$, vitamin C, salicylate, glutathione, uric acid also give this test positive.

Barfoed's Test

Reagent: Cupric acetate in acetic acid

Procedure: Take 5 cc of Barfoed's reagent and add 8 to 10 drops of unknown solution. Keep it in boiling water bath. Red precipitate. If red color appears in 0 to 5 min, it indicates monosaccharide. If red color appears in 10 to 12 min, it indicates disaccharides.

Principle: Copper reduction test in acid solution.

Note: This test is:

1. Not suitable for detection of sugar in urine or any fluid
2. Not specific for glucose
3. Simply detects monosaccharides
4. Negative with sucrose, as no free reducing group is present.
5. Glucose and fructose give this test positive
6. Disaccharides take longer time.

KOH Reduction Test

Procedure: Take 2 cc unknown solution, add 2 cc NH_3 and 0.5 ml 15 percent KOH, keep it in boiling water bath. It turns yellow-red on cooling, this test is specific for lactose.

Principle: It is a condensation reaction.

CHAPTER 13

Proteins

Learning objectives:

1. Describe different tests done for proteins.
2. Explain Protein colour reactions.
3. Discuss Biological value of proteins.
4. Illustrate principle of Protein precipitation.

Fundamental Role of Proteins in Life

The nature of living organisms depends essentially on two classes of substances: Proteins and nucleic acids.

While nucleic acids contain blue prints for hereditary characteristics, the structures of proteins enable them to act as the catalysts (enzymes), hormones, antibodies, coagulation factors, serving as building block units for subcellular, cellular and organic structures, carriers (of gases, metals, hormones vitamins, *etc.*), contractile elements (in muscles), maintenance of acid-base balance and osmotic pressure, *etc.*

In order to understand how these substances play such vital roles, understanding of the relationship between their structures and their biological functions is important.

General Properties of Proteins

All proteins are polymers of amino acids and all except two have an amino group attached to the carbon atom next to the carboxyl group (the α-position); *i.e.* they are α-amino acids.

Experiments on Proteins

a. Composition tests
b. Color reaction
c. Precipitation reaction

Proteins occur in both soluble and insoluble states in the body. In routine, tests on soluble proteins are performed in clinical chemistry laboratory in serum, urine and CSF samples.

Composition Tests

In addition to C, H and O, the proteins invariably contain nitrogen and generally sulfur. Elements such as I, Fe, Cu, and Zn are also occasionally present.

Some proteins yield only amino acids when hydrolyzed (simple proteins), whereas others produce amino acids plus other types of molecules (conjugated proteins).

General Composition Test

Heat some powdered egg albumin in a dry test tube in which a moist strip of red litmus paper and a piece of filter paper moistened with lead acetate solution is suspended.

As powder chars on heating, fumes of ammonia are evolved, turning the red litmus blue (indicating presence of N and H) and lead acetate paper is blackened (indicating presence of S).

Sulfur is present in proteins as cystine, cysteine, or methionine. Majority of proteins contain more methionine sulfur than cystine plus cysteine sulfur. Exceptions are keratins, insulin, certain serum albumins which contain all their sulfur in the form of cystine and cysteine.

Color Reaction of Proteins and Amino Acids

Because of their peptide structure and the presence of different amino acid groups in their molecules, proteins react with a variety of agents to form colored products.

Several of these color reactions of proteins are of importance in the quantitative detection and qualitative estimation of proteins and their constituent amino acids (Fig. 13.1).

Proteins used in these tests are the following:

1. *Albumin:* The albumin is soluble in water, coagulated by heat, and deficient in glycine. They are products of both plants and animals. Examples include egg albumin, serum albumin, lactalbumin of milk. Albumins may be prercipitated from solution by saturation with ammonium sulfate.
2. *Globulin:* The globulins are insoluble in pure water, but they are soluble in dilute neutral solution of salts and are heat coagulable. They are precipitated by half saturation with ammonium sulfate. Examples – ovoglobulin of egg yolk, serum, globulin, myosin of muscle.
3. *Albuminoids or scleroproteins*: They are the least soluble of all the proteins and constitute a very diverse groups of proteins. They are entirely animal proteins and are chief constituents of exoskeleton such as hair, horn, hoofs, nails, as well as of supporting and connecting fibrous tissues, and of the organic material of cartilage and bone.

Examples – keratin of hair, horn, hoof, nail, elastin of connective tissue and ligaments, collagen of bone, cartilages.

Gelatin

It results from treatment of collagen with boiling water or dilute acids and is sometimes classified as *scleroproteins*. Gelatin is a protein derivative and differs from collagen and keratin in having a much simpler physical structure and is being easily soluble and digestible.

It lacks tryptophan and lacks certain other amino acids like tyrosine and cystine. It is rich in arginine.

Casein

It is a phosphoprotein, and makes up a third of protein of human milk and five-sixth of protein of cow's milk. This is precipitated by half saturation with ammonium sulfate. It lacks sulfur-containing amino acids.

Peptones

They are secondary derived proteins. They are hydrolytic products of simpler proteins. They are soluble in water, are not coaguable by heat, and are not precipitated by saturation with ammonium sulfate. They are precipitated by phosphotungstic acid. They lack sulfur containing amino acids.

Complete hydrolysis of a natural protein:

Protein → metaprotein → proteose → peptone → peptides → amino acid

Mixtures of proteoses, peptones and peptides are prepared commercially and used for bacterial culture media.

Mucoproteins

Mucoprotein present in saliva is termed as mucin. Mucoproteins are conjugated proteins consisting of proteins combined with mucopolysaccharides such as hyaluronic acid and chondroitin sulfate. Water-soluble mucoproteins have been obtained from serum, egg white and human urine.

Also, mucoproteins are important constituents of ground substance of connective tissue. Several gonadotropic hormones FSH, hCG are mucoprotein.

Color Reactions

These reactions are due to a reaction between some one or more of the constituents, radicals or groups of the complex protein molecule and the chemical reagent or reagents used in any given test.

Ninhydrin Test

Principle

The amino groups of amino acids are very resistant to hydrolysis, but may be easily removed by oxidation. When amino acids are heated with ninhydrin, they are quantitatively deaminated.

This test is given by α-amino group containing compounds, based on the principle of oxidative deamination. During this reaction CO_2, NH_3, aldehyde with one carbon less than the amino acid is produced.

Ninhydrin (triketohydrindene hydrate) is a powerful oxidizing agent which reacts with all amino acid between pH 4 and 8 to give a purple colored compound. This reaction is also given by primary amines, NH_3 but without liberation of CO_2. Amino acids, proline and hydroxy proline react to produce yellow color. This reaction is very sensitive, ideal for detection of amino acids on chromatograms and their quantitation.

Procedure

To 1 ml of protein solution, add two drops of freshly prepared ninhydrin reagent. Heat to boiling for one to two minutes and allow to cool. A blue color develops, if the test is positive. This is known as Ruhemann's color complex.

CHAPTER 14

Milk Analysis

Learning objectives:

1. Explain physical and chemical analysis of milk.
2. Discuss Biological value of milk.

Milk is an important constituent of food. It contains carbohydrates, fats, proteins, minerals and vitamin.

Out of total protein content of milk—80% is casein (a conjugated protein) present as calcium caseinate, 20% is lactalbumin and lactoglobulin (human milk has less lactalbumin than cow's milk).

Casein is a conjugated protein, conjugated with Ca and PO_4 with isoelectric pH of 4.6. It is the only protein, which precipitates out at its isoelectric pH. It is a phosphoprotein (phosphoserine)

Carbohydrate

Only carbohydrate is lactose. Glucose and galactose are present in traces. Lactose facilitates absorption of calcium from gut (*Table 14.*1).

Mineral

- Rich in Ca and PO_4
- Ca content is 10 mg/100 ml human and in cow, Ca is 130 mg/100 ml.
- Phosphate is 13 mg/100 ml in human and 100 mg/100 ml in cow mil.
- Inorganic components include all essential minerals except F.
- Human milk is rich in oleic acid and bovine milk is rich in palmitic acid. Adulteration with water lowers specific gravity. Arachidonic acid content is higher in cow's than human milk
- Also Zn, Mn are present
- Low/deficient in iron and copper.

Fats

Milk contains low molecular weight fats (TG), also cholesterol and lecithin are present (more in buffalo than human milk).

Fat in milk is known as buffer fat and it is mostly composed of TG.

Specific gravity of milk (1.028-1.035) depends on fat content (inverse relation). Specific gravity of skimmed milk is less (1.037), more in buffalo than cow.

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pH

Six to seven when fresh. On standing, milk pH is acidic because streptococcus lactis and many other micro-organisms cause fermentation of lactose to lactic acid. pH is determined by methyl red, chlorophenol red and phenol red.

Vitamins

- Vitamin A is in the form provitamin, *i.e.*, carotene and carotenoids,
- Vitamin B_1, B_2, B_{12} and vitamin D are present in milk.
- Vitamin C is absent in milk.
- Nicotinic acid is more in human milk.
- In infants, there is adequate store of Fe up to 3 months of age. So after 5 months, top feeds should be added. Mother's Milk provides immunity to child.

Humanization of Cow's Milk

- Main difference between human and cow's milk is in fat and protein content
- Human milk has high lactose content than animal milk
- To 100 ml milk, add 100 ml boiled water plus a pinch of sugar.

Cow and buffalo milk contain large proportions of proteins and fats, so adding equal amount of water to buffalo milk, we get values of protein and fat nearer to human milk and on adding sucrose, carbohydrate content becomes equal to that of human milk.

Table 14.1: Various nutrients in milk (g%)

	Protein	*Fat*	*Carbohydrate*	*Ash*
Human	1.2	3.8	7.0	0.7
Cow	3.3	3.8	4.8	0.2
Buffalo	4.7	7.5	4.8	0.8

Colostrum

Colostrum is secretion of mammary gland during early phase of lactation (up to five days in human and 5 to 12 days in cattles). It is rich in protein and globulin, poor in sugar and fat.

It is heat coaguable because of high globulin content. It is rich in globulin which provides passive immunity.

It is yellow in color because of high vitamin B_{12} content.

Cheese

Cheese is prepared by warming milk with rennet (commercial preparation of rennin). Commercial cheese is obtained by rennin coagulation of milk to calcium paracaseinate.

Cheddar

It is also rennin coagulation of milk and then it is soured by a starter of lactic acid bacteria. *Swiss cheese* is also started in a similar manner.

Whey Protein

Proteins left in the solution, after curdling of milk are whey proteins, *i.e.* lactalbumin and lactoglobulin. When casein is converted to paracaseinate, proteose (whey protein) are released.

Physical Examination of Milk

- *Color*—usually white in color
- *Appearance*—opaque thick liquid
- *Odor*—characteristic odor which varies from species to species
- *Reaction*—neutral or acid to litmus pH 6–7
- *Specific gravity*—1.028–1.035, can be measured by lactometer.

Chemical Analysis (Table 14.1)

Curdling of milk: To 1 test-tube of milk, add half test-tube of distil water and half test-tube of 1 percent acetic acid. White precipitate appears, filter it. Or take equal amount of milk and acetic acid to curdle the milk.

- *Test for protein:* Proteins give ninhydrin, biuret test in milk precipitate portion. All amino acid except cysteine, cystine are present in precipitate (ppt), so lead acetate test is negative.
- *Test for calcium:* To 2 cc of filtrate, add few drops of potassium oxalate, white ppt of Ca oxalate appears.
- *Phosphorus (pneuman's test):* To 2 cc filtrate, add 1 cc concentrated nitric acid, boil, cool, then add 2 drops of 10% ammonium molybdate, appearance of yellow precipitate shows presence of phosphorus.
- *Lactalbumin:* Fill 2/3rd test tube with filtrate, add a drop of chlorphenol red [pH 5 (yellow) – 6.7 (red)] to filtrate (pH 4.6). Add Na_2CO_3 drop wise, to obtain pink color (pH 5.6 pink, pI). Do heat coagulation test in this filtrate. White ppt of protein (lactalbumin) appears on heating.
- *Lactose (KOH reduction test):* Take 2 cc filtrate, add 2 cc concentrated NH_3 (or NH_4OH) and 0.5 ml of 15% KOH. Keep it in boiling water bath for 5 min. It turns yellow which becomes orange on cooling.
- *Fat:* Take small amount of dry precipitate in a clean test tube, add 3 to 4 ml ethanol, warm it on flame. Filter it. To filtrate, add 1 ml water or directly add 1 ml water to it. Fat droplets will float on the surface.

CHAPTER 15

Urine Exercises

Learning objectives:

1. Describe characteristics of urine.
2. Describe chemical constituents of normal urine.
3. Explain different abnormal constituents of urine and different methods for estimating these constituents.

Structure of Kidney

The body contains two kidneys placed symmetrically on either side of the vertebral column in the lower abdomen. Each kidney can be divided into two major parts, the outer part—cortex, inner part—medulla.

The primary functional unit of kidney is *nephron*. Each kidney has one million nephrons. Glomerulus is a net of capillaries (afferent and efferent arterioles) lying in Bowman's capsule. The renal tubule has three sections—proximal convoluted tubule, loop of Henle, distal convoluted tubule from where they form the beginning of collecting duct. The collecting duct merges within medulla to form larger ducts in the papilla of kidney → renal pelvis → ureter → urinary bladder.

Primary functions of kidney

1. Removal of wastes (urea, creatinine, uric acid)
2. Retention of nutrients (protein, amino acids, glucose, Na, Ca, Cl, HCO_3) and water
3. Acid-base balance
4. Water and electrolyte balance
5. Hormone synthesis (erythropoietin, renin, vitamin D).

Formation of Urine

Glomerular basement membrane allows compounds whose molecular weight is less than 69000 daltons to filter out of the capillaries and into the Bowman's capsule. Blood cells and larger molecular weight compounds are retained in the vascular compartment so that the glomerular filtrate is free of protein.

The rate at which plasma is filtered by the glomerulus is called GFR (glomerular filtration rate). It is 125 ml/min. Approximately, 80% of fluid and electrolytes filtered by glomerulus are *reabsorbed* in proximal tubule by active and passive resorption. *Active secretion* of body wastes also occurs here.

In the loop of Henle, further reduction in volume and recovery of Na and Cl occurs. In distal convoluted tubule, Na is reabsorbed under influence of aldosterone and excess body acid is removed.

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The isotonic fluid leaving the distal tubule enters the collecting ducts and ADH (antidiuretic hormone) increases the permeability of collecting duct and prevents an excretion (or diuresis) of water. The final concentration of urine occurs in the collecting ducts. The rate of urine passing to bladder is 1 to 2 ml/min, while GFR is 125 ml/min.

Characteristics of Normal Urine

Examination of urine is now mostly performed
1. to screen for unsuspected disease
2. to confirm or eliminate diagnosis
3. to assist in treatment.

Physical Appearance

Normal urine is pale, straw-yellow because of presence of pigment urochrome mainly and uroerythrin and urobilin in small amounts. *Highly concentrated* urine has dark amber color, and its causes are sweating, diarrhea, heart failure. *Pale urine* is observed when concentration of solid decreases and volume is large. It is seen after excessive fluid intake, alcohol, diuretics, chronic renal failure, diabetes insipidus (specific gravity low). Pale urine of high specific gravity occurs in diabetes mellitus (Table 15.1).

Table 15.1: Abnormal color of urine

Constituent	*Color*
Bile	greenish yellow
Blood	red, reddish brown, smoky
Hemoglobin	deep red
Melanin, alkapton, porphyria	darken on standing
Myoglobin	dark red
Rifampicin and phenidione	orange

Odor (Smell)

- Normal odor of urine is aromatic (ammonical). In infants, it is strong ammonical. Following infection with *E. coli* it has, fishy odor. Ketone bodies in urine give it a fruity odor. In conditions like maple syrup disease, urine has a characteristic smell.

Specific Gravity

In general, specific gravity is a reflection of state of hydration and functional ability of kidney. Normal specific gravity of urine is 1.015 to 1.025. Specific gravity of urine increases as concentration of main constituents increases (*e.g.* Na, Cl, urea, creatinine, sugar, protein).

Low fixed (< 1.010) is observed in diabetes insipidus (DI), (Chronic renal failure) CRF.

High (> 1.030) occurs in DM, albuminuria, radiopaque dye.

pH

Normal pH of urine is 4.7 to 7.5

- pH of urine is alkaline after meals, vegetarian diet known as alkaline tide.
- After non-vegetarian diet pH of urine is acidic.
- Alkaline pH also occurs when urine contains bacteria in large number.
- pH is a function of need to eliminate fixed acids and urinary pH reflects status of the pH of the blood.

Need to manipulate pH

- In case of acidic urine, prevention of calcium carbonate and magnesium–ammonium phosphate kidney stones.
- Alkaline urine is produced to prevent oxalate, uric acid stones. Alkalinization of urine is occasionally used to treat drug overdose (*e.g.* Salicylate) or hemolytic transfusion reaction and for optimization of dosage of antibiotics.

Volume

Volume of urine is influenced by fluid intake and diet. Normal urine volume is 1 to 1.5 L/24 h. Polyuria occurs when urine volume is more than 1.5 L/24 hrs and is observed in DM, DI and late stage of CRF. The term oliguria is used when urine volume is less than 400 ml. Oliguria occurs in diarrhea, acute nephritis, early CRF, liver disease, congestive cardiac failure (CCF). When urine volume is less than 100 ml, it is termed as *anuria.* Anuria occurs in mercury poisoning, bilateral renal stone, shock.

Deposits

Urine is usually transparent and presence of phosphate and urate results in cloudiness of urine. Presence of phosphate gives rise to white/buff colored deposits which disappear on acidification of urine. Whereas, presence of urate results in buff pink colored deposits which disappear on heating. Pathological deposits in urine are pus, blood, bacteria resulting turbidity or thick deposits.

Chemical Constituents of Normal Urine

Normally, 50 to 60 g of solid constituents are present in urine over a 24 hours period. Organic substances are 30 to 35 g and are composed of urea, uric acid, creatinine mainly. Others include amino acids namely cysteine, leucine, tyrosine, drugs and their metabolites for instance bilirubin, cholesterol, indigo, xanthine.

Inorganic constituents are 20 to 25g. They include Ca, PO_4 , NH_4 ,$^+$ SO_4, Cl

1. *Urea:* 25 to 30 g/24 hr. Normally urea appears in urine.
   High protein diet, catabolic states result in increased urea excretion and excretion is reduced in impaired liver function, kidney disease.
2. *Creatinine*: Normally 0.5 to 1.0 g/24 hrs of creatinine is excreted in urine and rate of creatinine excretion depends on muscle mass, increased muscular activity, kidney disease, muscular dystrophy.
3. *Amino acids* 150 to 200 mg

CHAPTER 16

Cerebrospinal Fluid (CSF)

Learning objectives:

1. Describe all characteristics of Cerebrospinal fluid.

Cerebrospinal fluid is usually obtained for diagnostic purposes by lumbar puncture. CSF should be submitted separately for different studies namely:

a. Chemistry and immunology studies
b. Microbiological examination
c. Total and differential cell count
d. Cytology

The four major categories of diseases in which CSF examination is required are:

1. Meningeal infection
2. Subarachnoid hemorrhage
3. CNS malignancy
4. Demyelinating diseases

The following chemical tests are commonly carried out on CSF: Determination of glucose, chlorides and proteins, quantitative test for globulin

Appearance

Normal CSF is clear, colorless and gives no coagulum or sediment on standing under sterile conditions.

Color

Presence of blood is main cause of abnormal color. Normally, no RBCs are present in CSF and some may be introduced as a result of trauma while obtaining the fluid. Pathologically, blood may be present in subarachnoid hemorrhage and in hemorrhage into the ventricles.

Turbidity

It is seen when there is marked increase in the number of cells or when organisms are present in CSF. Commonly seen in streptococcal infection.

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Coagula

In pathological fluid, where there is increase in proteins, in CSF, fibrin clots may form.
In tuberculous meningitis, a fine web-like coagulum is sometimes formed when fluid is allowed to stand overnight.

Chemical Examination

Glucose

The sugar content of normal lumbar fluid is usually between 50 and 80 mg per 100 ml and is slightly lower than the blood sugar (*i.e.* 65% of blood glucose levels)
Most important pathological change is decrease in glucose levels which occurs in meningitis.

Estimation of Sugar

This may be carried out by any of the blood sugar methods in use.

Proteins

The protein content of normal lumbar fluid lies between 15 to 45 mg per 100 ml. The protein present is almost entirely albumin.
Increase in total protein is the commonest abnormality found in polyneuritis, meningitis, tumors.

Estimation of Protein

By turbidimetry method

Principle

Proteins are precipitated by 3 percent TCA and the resulting turbidity is read colorimetrically.

Procedure

Take 3 test tubes, and label them T, S, B.

	T (ml)	S (ml)	B (ml)
CSF	1	-	-
Standard	-	1	-
DW	-	-	1
3% TCA	3	3	3

Mix well, keep at room temperature for 10 minutes.
Read absorbance at 420 nm.

Calculation

$OD_T/OD_S \times 50$ mg/100 ml

Chlorides

Chloride content of normal CSF is 120 to 130 meq/L and is appreciably higher than plasma chloride.

Most important alteration is in meningitis where a decrease is observed and lowest values are seen in tubercular meningitis.

Estimation of Chloride

Methods used for plasma chloride can be used for CSF chloride estimation.

Questions:

1. Write the properties of Cerebrospinal Spinal Fluid.
2. What is the chloride content of normal CSF? Discuss the causes of its alteration.

Bibliography:

Burtis, C., Ashwood, E., Bruns, D. and Tietz, N., 2008. Tietz Fundamentals Of Clinical Chemistry. St. Louis, Mo.: Saunders Elsevier.
Kaplan, L. and Pesce, A., 2010. Clinical Chemistry. St. Louis, Mo.: Mosby/Elsevier.
Lehninger A, Nelson D, Cox M. Lehninger principles of biochemistry. New York: Worth Publishers; 2000.
Miller, 1., 2020. Handbook Of Specimen Collection And Handling In Microbiology. [online] Stacks.cdc.gov. Available at: <https://stacks.cdc.gov/view/cdc/7590>.
Murray, R., Bender, D., Botham, K., Kennelly, P., Rodwell, V. and Weil, P., 2012. Harpers Illustrated Biochemistry 29Th Edition. Blacklick: McGraw-Hill Publishing
Tietz, N., 1987. Fundamentals Of Clinical Chemistry. Philadelphia: W.B. Saunders.
Voet, D., Voet, J. and Pratt, C., 2016. Fundamentals Of Biochemistry. New York: Wiley.

CHAPTER 17

Enzyme Activity

Learning objectives:

1. Explain roles of different enzymes in body- Amylase, Pepsin, Renin, Trypsin.
2. Describe Starch digestion and Protein digestion.

Amylase

Saliva

Saliva is secreted by three pairs of glands:

Parotid
Submaxillary
Sublingual

These secretions vary in character, parotid saliva is watery, while secretions of other glands are higher in mucin and more viscid. Ordinary saliva is a mixture of the secretion of these glands and shows considerable variations in composition in different individuals and in same individual at different times. The secretion in saliva is result of reflex stimulation of secreted nerves through a center in medulla oblongata.

The amount of saliva secreted by an adult in 24 hours is variable : 1000 to 1500 ml.

Composition

Weak alkaline to weakly acidic in pH. pH range is 6.7 to 9, optimum pH is 6.6. Specific gravity of saliva is 1.007. It is composed of 0.7 percent solid matter which is 0.5 percent organic and 0.2 percent inorganic in nature.

Organic matter – 0.4 percent protein:
Chiefly mucin, with small amount of albumin and globulin
Enzymes: Chiefly salivary amylase
Metabolites: Urea, uric acid, cholesterol, vitamins, and phospholipids.

Salivary Amylase

Principal enzyme of saliva is salivary amylase. It is an amylolytic enzyme catalyzing the hydrolytic splitting of starch, glycogen and dextrin into simpler molecules.

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Amylases

Amylases are divided into α-*amylase,* β-*amylase* depending on the basis of different points of attack on oligosaccharides such as starch glycogen, amylopectin, amylose and dextrins.

α-*amylase:* It is of animal origin, and now frequently referred to as endoamylase. α-amylase hydrolyzes more interior linkages of the branched oligosaccharides (starch, glycogen, *etc.*) producing fragments larger than maltose. The attack is at α1, 4 glucosidic linkage : *Dextrinogenic* (produce dextrins). Both pancreatic amylase and salivary amylase are α-amylase.

β-amylase is of plant origin (*e.g.* malt-amylase). They hydrolyze oligosaccharides from non-reducing end of the chain at second α-1, 4 glucosidic linkage forming a molecule of β-maltose. It sequentially removes maltose units, and in branched substrates, the enzyme removes β-maltose molecules consequently, from non-reducing end and until a branch point, *i.e.* α-1, 6-glucosidic bond is reached. It produces maltose: *Saccharogenic*.

Action of Amylase on Hydrolysis of Starch

Starch is of two types : i) Amylose ii) Amylopectin

Amylose contains glucose units in α-1, 4 glucosidic linkage in a straight chain.

Amylopectin contains chains of glucose units like those of amylose and has branches of these glucose linked through α-1, 6 glucosidic linkages.

α-amylase first acts upon amylose to split more central linkage forming mixture of smaller dextrins, but, only a little maltose.

First product of action of amylase on starch is soluble starch (gives blue color with iodine). Further action of amylase on starch consists of dextrins and maltose. The dextrins differ in molecular size and complexity, depending upon extent of amylase action.

The higher members of the series resemble starch in giving blue or purple color (amylodextrin) with iodine, as the molecule becomes smaller by the splitting of maltose, a red color is given with iodine (erythrodextrins) and lower members of series give no color at all with iodine (achroodextrins).

Thus, during the action of salivary α-amylase on starch, free maltose is produced almost immediately and progressively increase in amount; at the same time the color reaction with iodine changes from blue through red to colorless (Fig 17.1).

Both the increase in reducing sugar and the change in the iodine color reaction are used in the following course of action of amylase on starch (Fig. 17.2).

Salivary amylase acts at an optimum pH of 6.6. Electrolytes have an important influence upon the action of amylases, *e.g.* Cl^-, Br^- and NO^{3-}. ions have a pronounced stimulating action upon salivary amylase.

1. Removal of chlorides from saliva by dialysis renders the amylase inactive
2. Amino acids, particularly asparagine have an accelerating action.
3. Salts of heavy metals such as silver and mercury inhibit this enzyme.

Because of sensitivity to acid, salivary amylase ceases to act in the stomach as soon as food reaches stomach.

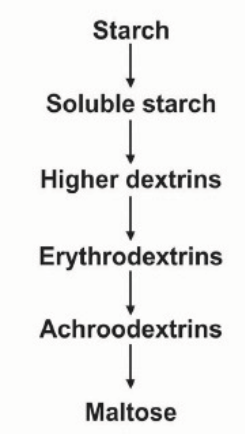

Fig. 17.1: Stages of digestion of starch

Experiment

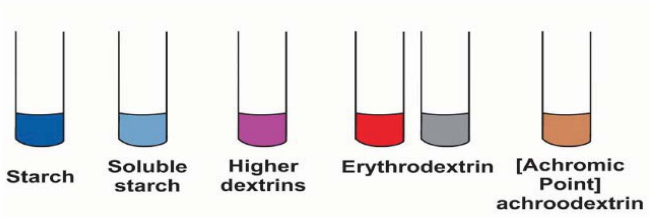

Fig. 17.2: Hydrolysis of starch

To demonstrate activity of salivary amylase:

1. *Collection of saliva:* Saliva is obtained by rinsing mouth with 20 cc distilled water in mouth and pour it in a beaker.
2. *Preparation of reaction mixture:* Take 5 ml starch solution, add 1 ml NaCl, 2 ml phosphate buffer (6.8). Keep it in water bath at 37°C, add 2 ml of salivary amylase to it.
3. Arrange 15 test tubes in a test tube stand, put 2 cc iodine solution to each test tube. Add 5 drops of reaction-mixture to first test tube, keep on adding 0.5 ml of reaction mixture at an interval of one minute to test tube till red color appears.

Observations

In first tube, a blue color is observed, in further tubes purple and then red color and no color are observed on successive addition of reaction mixture.

Note the *achromic point* where no color develops (10–12 tubes).

Achromic period: Note the tube number where no color appears, that tube number minus one gives achromic period.

Section V

Clinical Chemistry

CHAPTER 18

Urea Estimation

Learning objectives:

1. Describe various methods of urea and creatinine estimation, their clearance properties.
2. Explain the clinical significance of blood urea levels in health and disease.

Introduction

Urea is the main end product of protein metabolism in the body. Removal of amino groups from amino acids, occurs from which urea is formed. Process of urea formation takes place in the liver.

Determination of blood urea is important not only in many diseases of the kidneys but in a wide range of conditions which are not primarily renal.

Experiment

Estimation of Blood Urea by Diacetyl Monoxime Method

Principle

Diacetylmonoxime (DAM) method: Urea reacts with DAMO (diacetylmonoxime) to give azo compound which is yellow in color and stabilizes color, intensifies by adding ferric alum and color is read at 430 nm.

Procedure: Prepare protein free filtrate by taking 0.2 ml of oxalated blood, 1.9 ml 5 percent Zn SO_4 and 1.9 ml 0.3 N NaOH and centrifuge (Fig 42.1).

	T	S	B
	(ml)	(ml)	(ml)
PFF	2	-	-
Working urea standard (0.025 mg/ml)	-	2	-
Distil water	-	-	2
DAM	2	2	2
Ferric alum	2	2	2
Mix			

Keep in boiling water bath for 5 minute. Cool it under tap water and take absorbance at 420 nm (Fig. 18.1).

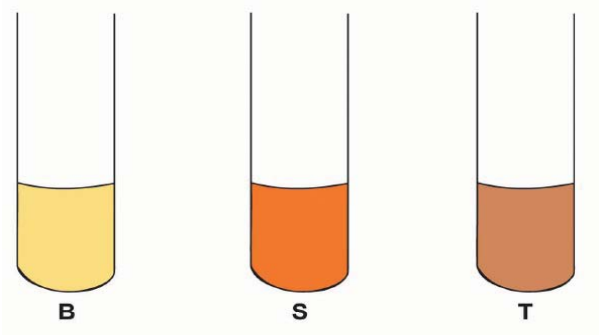

Fig. 18.1: Estimation of blood urea

Reaction

DAMO + water → hydroxylamine + Diacetyl

↓

Diazine + water derivative

Calculation

Concentration of Test

= ODt/ODs x 0.05/0.01 x 100 in dl
= ODt/ODs x 50 mg percent
4 ml (total vol PFF) has 0.02 ml blood

1 ml has 0.02/4
2 ml has 0.02 /4 x 2 = 0.1 ml blood

Working standard concentration is 0.025 mg/ml
i.e. 1 ml has 0.025 mg urea
2 ml has 0.025 x 2 = 0.05 mg urea 100
ml has 0.05 x 100 = 0.05 mg urea
Normal urea levels: 15 to 35 mg /dl
Merits of this method:
Urea reacts readily with DAMO
It is quick, DAMO is stable
Demerit:
Color developed is photosensitive
Not specific

Other Methods

Urease Method

$$Urea + H_2O \xrightarrow{urease} NH_3 + NH_2COOH \text{ (Carbamic acid)}$$

↓

$$NH_3 + CO_2$$

This ammonia is measured

Urease

Urostat dry chemistry: Strips for urea estimation in serum or blood.

Berthelot Reaction

NH_3 formed by urease reaction reacts with phenol in presence to hypochlorite to form an indophenol which with alkali gives blue colored complex and sodium nitroprusside acts as catalyst.

Glutamate Dehydrogenase Method (GDH)

$$\text{Urea + water} \xrightarrow{\text{Urease}} NH_3 + CO_2$$

$$\alpha\text{-ketoglutarate} + NH + NADH \xrightarrow{GDH} \text{L-glutamate} + H_2O$$

Nesselerization

NH_3 formed by urea reaction (reacts with Nessler's reagent) is determined colorimetrically.

Drawback of Methods Measuring Ammonia

They are susceptible to contamination of ammonia from lab and endogenous ammonia in the specimen.

Clearance

Clearance (CL) is ml of plasma cleared off from a particular solution in one minute by both the kidneys. Expressed as ml/min

$$CL = \frac{uv}{p}$$

u = urine urea
v = rate of urine flow
p = plasma urea
Normal rate of urine flow is 1 ml/min
Standard urea clearance:
when v < 2 ml/min.

$$CL = \frac{u\sqrt{v}}{p}$$

Normal range = 40 to 65 ml/min
Average value = 54 ml/min

CHAPTER 19

Creatinine

Learning objectives:

1. Describe different methods of determination of Creatinine.
2. Discuss Creatinine Clearance.

Creatine, methylguanidinoacetic acid is synthesized in the liver, passes into circulation and is taken up almost entirely by skeletal muscle for conversion to creatine phosphate.

Creatine is synthesized from three amino acids namely, glycine, arginine and methionine. Firstly, glycine and arginine undergo transamination in kidney to guanidoacetate and ornithine. Guanidoacetate undergoes methylation by S-adenosyl methionine in liver to form creatine. Creatine is stored in muscle as creatine phosphate.

About two percent of creatine is converted to creatinine. Creatinine is anhydride form of creatine. 24 hours excretion of creatinine is constant in an individual and is proportional to muscle mass and does not depend on dietary intake. Free creatinine is a waste-product of creatine and is present in all body fluids and creatinine is freely filtered by glomerulus.

Determination of Creatinine

1. Chemical method
2. Enzymatic method
3. Autoanalyzer
4. HPLC

Chemical Method: Jaffe's Method

Principle

Creatinine reacts with saturated alkaline picrate solution to produce a yellowish red colored complex, intensity of which is read at 520 nm.

This test is not specific because other non-creatinine chromogens in blood react similarly with alkaline picrate solution which are 20 percent in serum and 5 percent urine and raise the true value of creatinine by 0.2 to 0.4 mg percent.

Non-creatinine chromogens: Acetone, acetacetate, pyruvate cephalosporins, guanidine.

Substances such as glucose, ascorbate, aspartate, histidine cause fading of color of test.

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Modified Jaffe's Reaction

Lloyd's reagent—hydrated aluminium silicate (Fuller's earth)
Protein is precipitated with tungstic acid and supernatant fluid is mixed with Lloyd's reagent which adsorbs creatinine in acid condition. After centrifugation, pellet is formed which is resuspended in alkaline picrate to elute creatinine.

Procedure

Take 1 ml serum + 1 ml D/W + 1 ml 5 percent sodium tungstate + 1 ml 2/3 NH_2SO_4 mix, centrifuge, take supernatant.
Take three test tubes and label them as test (t), standard (s) and blank (B)

	T	S	B
	ml	ml	ml
PFF	2	-	-
Standard (0.01 mg/ml)	-	1	-
Distil water	2	3	4
5% NaOH	1	1	1
Saturated picric acid	1	1	1

Mix, keep at room temperature for 15 minutes and read at 520 nm (Fig. 19.1).

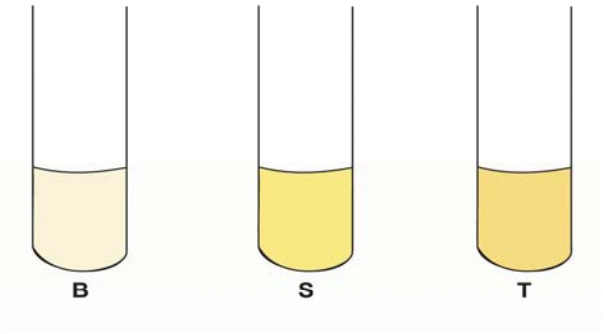

Fig. 19.1: Estimation of S. creatinine

Calculation:

$\frac{T}{S} \times 2$ mg percent
4 ml PFF has 1 ml serum
1 ml of PFF has ¼ ml
2 ml in test has $\frac{1}{4} \times 2 = 0.5$ ml sera
Concentration of standard being 0.01 mg per ml

$\frac{T}{S} \times \frac{0.01}{0.5} \times 100$ mg/dL
Normal value: 0.8–1.5 mg /dL

Urine Creatinine

Normal values: 100–200 mg/dL
1–2g/24 hours

Serum creatinine estimation follows Lambert-Beer's law till 15 mg percent

In case of urine, creatinine, we cannot apply it to the same concentration, so 1:100 dilution is done.

Procedure: Take three test tubes, label them as test (T), standard (S) and blank (B)

	T	S	B
	ml	ml	ml
Urine	3	-	-
Distil water	-	-	3
Standard	-	3	-
5% NaOH	1	1	1
Saturated picrate	1	1	1

Mix, take reading at 520 nm

Calculation:

100 ml of solution contains 1 ml urine
and 3 ml will contain:

$$\frac{1}{100} \times 3 = 0.03 \text{ ml}$$

$$\textit{Concentration of test} \quad \frac{0.01 \times 3}{0.03} \times 100$$

$$= \frac{T}{S} \times 100 \text{ mg/dL}$$

Interpretation

Creatinine is endogenously produced and released into body fluids at constant rates and its plasma levels are maintained within normal limits. Its clearance can be measured as an indicator of GFR.

Increased Creatinine Levels:

1. Renal disease
2. Due to:
   - Changes in muscle mass
   - Starvation
   - Muscle wasting diseases
   - After surgery
   - Corticosteroids administration
   - Physiological increase in GFR occurs in pregnancy

CHAPTER 20

Uric Acid

Learning objectives:

1. Discuss methods for estimation of serum Uric acid.
2. Interpret reasons for increased or decreased uric acid.

Uric acid (UA) is the end-product of purine metabolism. It is derived endogenously from break-down of tissue nucelic acids and exogenously from metabolism of purines taken in the diet. Total body miscible uric acid pool is 1.2 g of which 1/6th is in blood and rest is in tissues. There is a daily turnover of 600 mg of which 75 percent is excreted in urine and 25 percent is destroyed by bacteria in colon. In kidney, UA is filtered at glomerulus fully, reabsorbed in PCT (proximal convoluted tubules), so that UA is excreted in DT (distal tubules).

Normal values:

In males: 2.5–7.0 mg/dL
In females: 1.5–6.0 mg/dL

Estimation of Serum Uric Acid

Chemical Method

Principle

It is based on reducing property of UA. UA reacts phosphotungstic acid in alkaline medium. This oxidizes urate to allantoin and itself gets reduced to tungsten blue, absorbance of which is read at 660/ 710 nm

Phosphotungstic acid + UA + O_2 → Tungsten blue + Allantoin + H_2O_2 + CO_2

Demerits

1. Not specific for uric acid
2. This test can be given by non-urate chromogen

Preparation of Protein-free Filtrate (PFF)

Take 3.5 ml distil water (DW) and 0.5 ml serum. To this add 0.5 ml 10 percent sodium tungstate and 0.5 ml 2/3 N sulfuric acid. Mix, centrifuge at 4000 rpm for 5 minutes and take the supernatant.

Procedure

Take three test tubes and label them as T, S & B.

	T(ml)	S(ml)	B(ml)
PFF	3	-	-
DW	-	3	-
UA Standard (0.01 mg/l)	-	-	3
14% Na_2CO_3	1	1	1
Phosphotungstic acid	1	1	1

Mix. Let it stand for 15 minutes. Set zero with bank and read at 700 nm (Fig. 20.1).

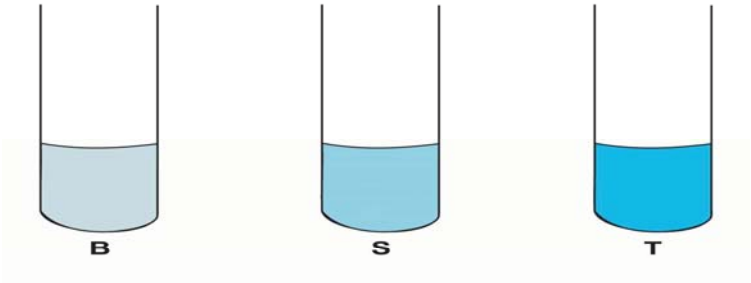

Fig. 20.1: Estimation of S. uric acid

Precautions

1. pH of ppt should be below 3 to avoid loss of UA.
2. pH for color development should be alkaline.

Calculations

1. Concentration of working standard is 0.01 mg/ml.
   In 3 ml, it is 0.03 mg/ml.
2. Total 5 ml solution for PFF has 0.5 ml serum, 3 ml solution will have

$$\frac{0.5}{5} \times 3 = 0.3$$

$$\frac{T}{S} \times \frac{0.03}{0.3} \times 100$$

$$= \frac{T}{S} \times 10 \text{ mg/dL}$$

Disadvantages

1. Some UA is carried down with protein during precipitation
2. Turbidity can occur

3. Beer's law is followed up to 9 mg /dL
4. Interfered by theophylline—false positive results

Enzymatic Method

Uricase Method

Uricase

Uric acid + $2H_2O + O_2$ - - - - → Allantoin + H_2O_2 + CO

H_2O_2 + 4-amino antipyrine – – – – → quinone red

Quinone red is read at 550 nm.

Uricase method can be automated, and dry chemistry and HPLC methods are also possible.

Interpretation

1. *Decreased uric acid*:
   After corticosteroid, ACTH administration
   Drugs:
   Aspirin
   Probenecid
   Penicillamine
   Indomethacin
   Allopurinol
2. *Increased uric acid*:

Defective Synthesis/Excretion Defective

1. Increased PRPP synthetase activity: Resulting in purine
   Overproduction and excretion
   Increased activity
   Resistance to feedback inhibition
   Low km for ribose 5 P
2. HGPRTase partial deficiency (resulting in decreased utilization)
3. Excretion: Tubular secretion defect

Gout

pK of UA is 5.8 and normal UA and its monosodium salts are present in body fluids in soluble form.

In hyperuricemia, serum UA exceeds solubility limit, causing crystallization of sodium urate in soft tissues and joints forming deposits called tophi. Tophi causes an inflammation reaction in joints termed as *Gouty arthritis*.

CHAPTER 21

Glucose (Sugar)

Learning objectives:

1. Explain different methods for measuring Blood Glucose.
2. Describe Glucose Tolerance Test.

Estimation of Blood Sugar O-toluidine Method (Table 21.1)

Experiment

Principle

Glucose condenses with o-toluidine in glacial acetic acid when heated at 100°C, produces an equilibrium mixture of n-glycosylamine and corresponding Schiff 's base to produce a blue green product (chromogen), absorbance of which is measured at 630 nm.

Sugar + O–toluidine - - - → glycosylamine

↑↓

Green chromogen ←- - - Schiff 's base

Procedure

Take 0.2 ml serum sample and add 1.8 ml of 4 percent TCA. Mix, centrifuge. Take 0.5 ml of supernatant (PFF). Take three test tubes label them as test (T), standard (S) and blank (B).

	T	S	B
PFF	ml	ml	ml
Sugar standard	0.5	-	-
(0.1 mg/ml)	-	0.5	-
Distil water	-	-	0.5
O-toluidine reagent	2.5	2.5	2.5

Keep the tubes in boiling water bath for 9 minutes and cool it under cold water for 5 minutes. Then read OD at 630 nm (color is stable for 1 hr) (Fig 21.1).

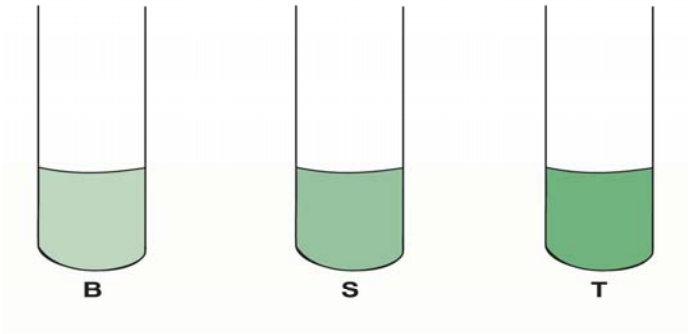

Fig. 21.1: Estimation of B sugar

Calculation

Standard concentration:

1 ml standard has 0.1 mg sugar

0.5 will have $0.1 \times 0.5 = 0.05$ mg/ml sugar

PPF: 0.2 ml serum is taken in 2 ml of PFF

$$0.5 \text{ ml will have } \frac{2}{0.2} \times 0.5 = 5 \text{ mg/ml}$$

$$\text{Concentration of sugar in T} = \frac{\text{ODT}}{\text{ODS}} \times \frac{0.05}{0.05} \times 100 = \frac{\text{T}}{\text{S}} \times 100 \text{ mg/dL}$$

Precautions

1. O-toluidine reagent is highly corrosive and should not be pipetted by mouth.
2. Store O-toluidine in amber colored bottle.

Advantages

1. Sensitive and only galactose produce absorbance comparable with that of glucose which is present in negligible amount in blood normally.
2. Hemolysis does not interfere with the test.

Disadvantages

1. Bilirubin contributes to some increase in glucose values and should be read with caution in neonatal jaundice.
2. In uremia, higher values are obtained.
3. Thymol preservatives inhibit color formation.
4. Dextrin causes turbidity in final color
5. Presence of EDTA >1 mg/ml or NaF >5 mg/ml cause increase in color.

Other chemical methods - 1. Folin Wu
2. Nelson Somoyogi
3. Inverse colorimetry

Enzymatic Methods

Glucose Oxidase Method – (GOD – POD Method)

Principle

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Peroxidase}} H_2O + [O]$$

$$[O] + \text{acceptor} \longrightarrow \text{colored chromogen}$$

Acceptor

O – dianisidine
O – toluidine
4 – aminophenazone (used commonly)
4 – aminoantipyrine.

Advantages

1. No interference by reducing agents such as uric acid, creatinine, GSH, Hb
2. Less interference by drugs like INH, paracetamol, hydralamine, tolazamide
3. Ascorbate retards the color to a lesser extent
4. Minor interferences are caused by xylose, galactose, L-dopa.

Hexokinase Method

Principle

$$\text{D-glucose} + ATP \xrightarrow{\text{Hexokinase}} \text{G-6-P} + ADP$$

$$\text{G-6-P} + NAD \xrightarrow{\text{G6PD}} 6\text{ gluconolactone} + NADH + H^+$$

Increase in absorbance of NADH is measured at 340 nm which is proportional to amount of glucose.

Advantage

It is unaffected by uric acid, bilirubin, vitamin C, lipemia, hemolysis.
But, thimerosal (anticoagulant) interferes with this test.

CHAPTER 22

Bilirubin

Learning objectives:

1. Explain detailed experiment for estimation of total serum bilirubin.
2. Enumerate all other methods for bilirubin estimation.
3. Discuss classification of Jaundice.

Bilirubin is formed from Hb in RES (reticuloendothelial system) and circulates attached to plasma albumin, in low concentration in the blood.

Bilirubin is insoluble in water and conjugated bilirubin is excreted in bile as diglucuronide and thus made water-soluble.

Experiment: Estimation of Total Serum Bilirubin

Principle

Bilirubin reacts with diazotized sulfanilic acid to give a purple color at neutral pH, absorbance of which is measured at 540 nm

Methanol accelerates diazotization process.

This reaction is termed as *van den Bergh reaction*

i. *Direct-acting bilirubin* or *conjugated bilirubin* is water-soluble and reacts with diazo reagent within one minute.
ii. *Indirect-acting bilirubin* or *unconjugated bilirubin* is non-polar, insoluble in water and does not react with diazo reagent.
iii. Indirect bilirubin = Total bilirubin – Direct bilirubin.

Reagents

Diazo A—sulfanilic acid in HCl

Diazo B—$NaNO_2$

Diazo reagent—10 ml of A and 0.3 ml of B should be prepared freshly

Diazo blank—HCl (0.1 N)

Procedure

Total Bilirubin

Take 2 test tubes, label them as test (T) and control (C)

	T(ml)	C (ml)
DW	1.8	1.8
Serum	0.2	0.2
Diazo reagent	0.5	-
Diazo blank	-	0.5
Methanol	2.5	2.5

(Addition of methanol dissolves un congrugated bilirubin)
Mix and keep item dark for 30 min
Read at 530 nm and take OD of standard as 0.24 (Fig 22.1)

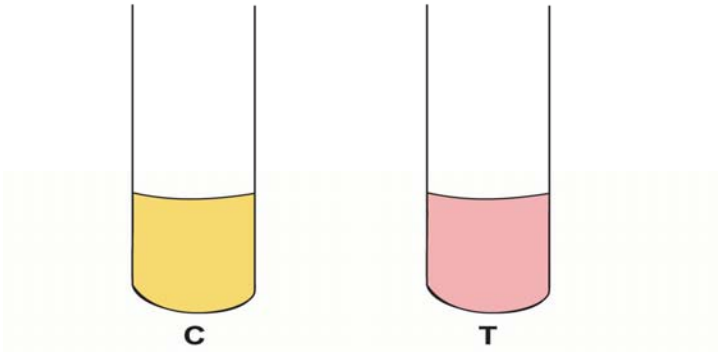

Fig. 22.1: Estimation of S. bilirubin

Calculation

$$\text{Serum bilirubin (mg/dl)} = \frac{OD_T - OD_C}{OD_S} \times 8$$

$$= \frac{T - C}{0.24} \times 8 \text{ mg/dl}$$

Bilirubin Standard

Artificial standard: Methanol red in glacial acetic acid is used.
OD of methyl red is 0.24 which corresponds to 8 mg /dL of bilirubin.

Precautions

1. Hemolyzed sample should not be used.
2. Color is photosensitive, so keep it in dark, take reading immediately.

3. Interfering substances are:
   - Vitamin B complex
   - Carotene
   - Hemolysis

Other Methods

Direct Bilirubinometer

Absorbance of bilirubin in serum at 454 nm is proportional to its concentration.
(Here second filter at 540 nm is chosen as oxy Hb has same absorbance at 454 nm. (Oxy Hb gives absorbance at 454 and 540 nm while bilirubin reads at 454).
This method is used for neonates.

Reflectance Spectrophotometry

This is a thin film technique where sodium benzoate and caffeine is present in top layers. The second layer has gelatin to trap serum protein and third layer has cationic polymer called a *mordant* that binds bilirubin. It is read at 400 nm. It is useful in neonates with physiological jaundice.

HPLC

Interpretation

Jaundice—It is yellow discoloration of tissues due to bilirubin deposition with raised S bilirubin (BR) levels.

- *Normal values of bilirubin*: 0.2 to 0.8 mg/dL
- *Latent jaundice*: When serum bilirubin levels are 1 to 2 mg/dl
- *Clinical jaundice*: When levels are >2 mg/dl

Classification of Jaundice

- Prehepatic/hemolytic
- Hepatic
- Posthepatic/obstructive

Prehepatic

- It is due to increased breakdown of Hb
- Liver cells are unable to conjugate all the increased BR formed.

Causes

- Hemoglobinopathies
- Hereditary sphereocytosis

CHAPTER 23

Proteins

Learning objectives:

1. Describe determination of total proteins in serum by Biuret method.
2. Interpret different causes of hypoproteinemia and hyperproteinemia.

Determination of Total Proteins in Serum by Biuret Method

Principle

Biuret method depends on the presence of peptide bonds in all proteins. These peptide bonds react with cupric ions in alkaline solutions to form a violet colored product/complex, whose absorbance is read spectrophotometrically at 540 nm. This test derives its name from the fact that when urea is heated to 180° C in alkaline medium responds to the test.

In the biuret reaction, a colored chelate is formed between Cu^{+2} ions and carbonyl oxygen and amide nitrogen atoms of peptide bonds. One copper ion is probably linked to six nearby peptide linkages by coordinate bonds.

Reagent

Quantitative biuret reagent:
$CuSO_4$ gives Cu^{+2} ions
NaOH is a strong alkali
Sodium potassium tartarate: Stabilizing agent
Iodide: Acts as antioxidant, complexes copper ions, otherwise Cu oxidizes to CuO which precipitates. Also, it maintains their solubility in alkaline solution.

Procedure (Fig. 23.1)

Take three tubes, label them as test (T), standard (S) and blank (B).

	T (ml)	S (ml)	B (ml)
DW	2.4	-	2.5
Working std (280 mg/dL)	-	2.5	-
Serum	0.1	-	-
Quantitative biuret reagent	3.5	3.5	3.5

Mix, keep in water both at 37°C x 10 min
Take OD at 540 nm (Fig 23.1)

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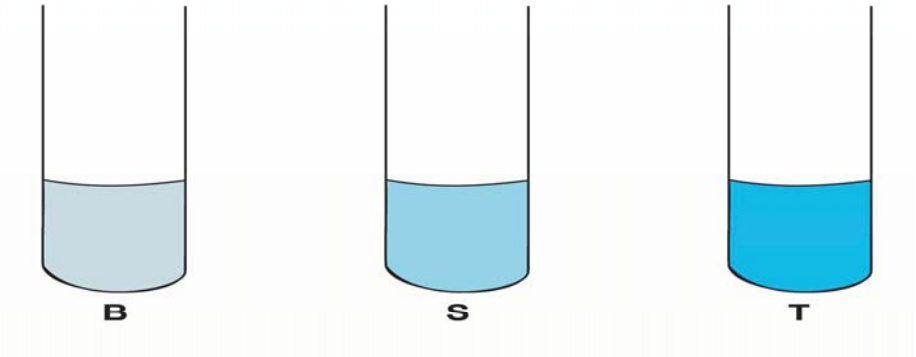

Fig. 23.1: Estimation of S. proteins

Calculation

$$\frac{OD_T}{OD_S} \times 7 \text{ g/dL}$$

Standard = 280 mg/100 ml = 2.8 mg/ml = 7 mg/2.5 ml
Serum used is 0.1 ml

$$\frac{OD_T}{OD_S} \quad \times\frac{7}{0.1}\times \quad \frac{100}{1000} = \frac{T}{S} \times 7\text{g/dL}$$

Normal range

Total proteins: 6.3 to 8.3 g/dL
A/G = 1:2
Albumin: 3.7 to 5.3 g/dL
Globulin: 1.8 to 3.6 g/dL
Most plasma proteins with the exception of Ig and protein hormones are synthesized in liver.
Albumin is most abundant plasma protein accounts for one-half of plasma protein mass.

Applied Aspects

Hypoproteinemia

(Low levels of proteins in blood)

Causes

1. *Decreased intake*:
   Malnutrition
   Malabsorption
2. *Decreased synthesis:*
   Primary
   Analbuminemia

Acquired
- Chronic
- Liver disease
- Rheumatoid arthritis

3. *Increased excretion/catabolism*:

Excretion

Renal: Loss of albumin in urine in nephrotic syndrome

GIT
- Protein losing enteropathy
- Ulcerative lesions

Skin
- Burns
- Exudative lesion
- Pemphigus
- Psoriasis
- Hemorrhage

Catabolism

Severe sepsis
Fever
Trauma
Malignant disease

Increased volume of distribution (hemodilution)

Overhydration
Sample from I/V site
Increases capillary permeability: septicemia

Hyperproteinemia

Increase in protein concentration

Causes

1. *Decreased volume of distribution:*
   Dehydration (hemoconcentration)
2. *Increased protein synthesis:*
   a. Hypergammaglobulinemia
   b. Polyclonal
      i. Chronic infection:
         Tuberculosis
         Leprosy
      ii. Cirrhosis
      iii. SA (sarcoidosis)

CHAPTER 24

Alkaline Phosphatase (ALP)

Learning objectives:

1. Explain estimation of Alkaline phosphatase.
2. Evaluate clinical significance of ALP.

Alkaline phosphatase is found in many tissues including bone; liver, intestine, kidney, placenta.

Estimation of ALP

Principle

4 nitrophenyl phosphate in presence of ALP is converted to 4 nitrophenoxide (colorless) at pH 10. Phenol released by enzymatic hydrolysis from phenyl phospate is estimated colorimetrically.

Procedure (Fig. 24.1)

Take 4 test tubes, label them
test (T), control (C), standard (S), blank (B)

	T (ml)	C (ml)	B (ml)
Buffer (pH 10)	1	1	1
PP substrate (Disodium phenyl phosphate)	1	1	1

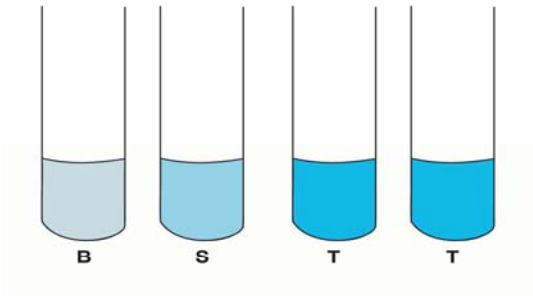

Fig. 24.1: Estimation of ALP

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Incubate in water bath at 37°C for 3 min

	T	C	S	B
Phenol standard (10 ug/ml)	-	-	1	-
DW	-	-	-	1
Serum	0.1	-	-	-
Mix gently, incubate at 37°C for 15 minutes				
Serum	-	0.1	-	-
0.5 NaOH	0.8	0.8	0.8	0.8
0.5 N $NaHCO_3$	1.2	1.2	1.2	1.2
Aminoantipyrine	1	1	1	1
Potassium ferricyanide	1	1	1	1

Read the color at 510 nm
Functions of reagents:
NaOH – to stop the reaction
$NaHCO_3$ – provides alkaline medium
Aminoantipyrine – gives color

Calculation

Amount of phenol in standard tube is 10 mg. Thus, phenol produced in 15 minutes in test will be
T–C/S–B x 10 mg
= T–C/S–B x 100/0.1 x 100 μg/100 ml
Hence, 100 ml of serum would liberate
T–C/S–B x 10 mg of phenol
Since 1 KAU is the production of mg of phenol in 15 minutes.
Under the condition of test therefore serum ALP = T–C/S–B x 10 KAU/100 ml
Normal range = 3–13 KAU/100 ml

Clinical Significance

ALP is elevated in:
Cholestatic liver diseases
Paget's diseases of bone
Cirrhosis
Bone tumors

Isoenzymes

Placental – heat-stable
Hepatic – heat-labile
Bone – heat-labile
(details are discussed under chapter: Liver Function)
Separation of isoenzymes:
1. Electrophoresis
2. Immunological techniques

Questions:

1. Write the principle of estimation of Alkaline Phosphatase. What is its clinical significance?
2. What are isoenzymes?

Bibliography:

Burtis, C., Ashwood, E., Bruns, D. and Tietz, N., 2008. Tietz Fundamentals Of Clinical Chemistry. St. Louis, Mo.: Saunders Elsevier.
Kaplan, L. and Pesce, A., 2010. Clinical Chemistry. St. Louis, Mo.: Mosby/Elsevier.
Lehninger A, Nelson D, Cox M. Lehninger principles of biochemistry. New York: Worth Publishers; 2000.
Miller, 1., 2020. Handbook Of Specimen Collection And Handling In Microbiology. [online] Stacks.cdc.gov. Available at: <https://stacks.cdc.gov/view/cdc/7590>.
Murray, R., Bender, D., Botham, K., Kennelly, P., Rodwell, V. and Weil, P., 2012. Harpers Illustrated Biochemistry 29Th Edition. Blacklick: McGraw-Hill Publishing
Tietz, N., 1987. Fundamentals Of Clinical Chemistry. Philadelphia: W.B. Saunders.
Voet, D., Voet, J. and Pratt, C., 2016. Fundamentals Of Biochemistry. New York: Wiley.

CHAPTER 25

Calcium and Phosphorus

Learning objectives:

1. Discuss methods for measuring total Calcium and Phosphorus.
2. Interpret causes for increase or decrease of Calcium and Phosphorus.

Measurement of Total Calcium

Principle

O-cresolphthalein complexone method (OCPC)
Metal complexing dye CPC [3', 3" – bis (biscarboxy methyl) amino methyl 5', 5" – dimethylphenophthalein] forms a red chromophore with calcium ions in alkaline solution, it is generally measured at 578 nm (wavelength between 570 and 580 nm).

Chromogen reagent has:

1. 8 hydroxyquinoline to mask interfering cations mainly Mg.
2. Urea to eliminate turbidity of lipemic serum and to enhance complex formation.
3. Ethanol to prevent color development in blank.

Chromogen Reagent

Prepare fresh reagent by taking equal amount of OCPC and diethamolamine.

Procedure

Take three tubes label them T, S, B (Fig. 25.1).

	T	S	B
OCPC reagent (ml)	5	5	5
Standard (10 g%)	-	-	-
Serum	50 ul	50 mL	-
Mix			
Read the absorbance at 570 nm (Fig 49.1).			

Calculation

S. calcium = OD_T/OD_5 x 10 in mg /dL
Normal range = 9 to 11 mg /dL

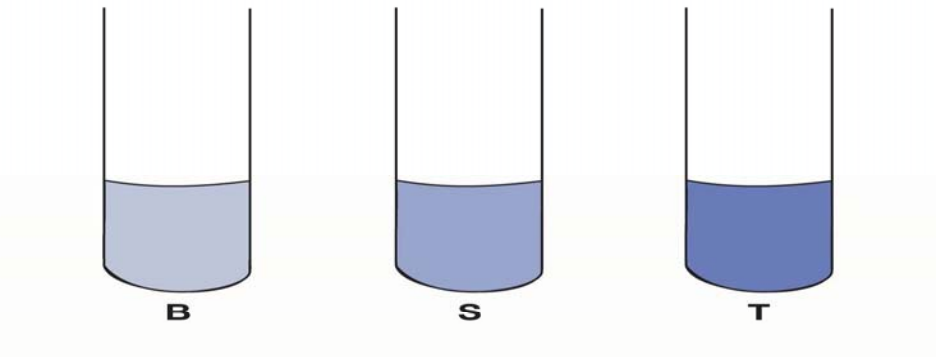

Fig. 25.1: Estimation of S. calcium

Precautions

1. Do not use hemolyzed sample.
2. All glassware used in the determination should be acid-washed and thoroughly rinsed with distilled water.
3. Avoid venous stasis during venipuncture.

Other Methods

1. *Titration method (Clark and Collip):* Calcium is precipitated as oxalate directly from the serum and after washing, the precipitate is dissolved in acid and titrated with permanganate.
   Disadvantage
   Time consuming (end-point is not clear).
2. *Iodometric titration:* An excess of permanaganate is used, potassium iodide is added and iodine liberated from this excess is titrated with 0.025 N thiosulfate using starch as indicator.
   Drawback: Not very accurate.
3. *Titration with EDTA:* In alkaline medium Ca is titrated with ammonium phosphate.
   Drawback – End point is vague.
4. *Flame photometer:* Ca and Mg are more difficult to be excited in flame than Na and K. Consequently, a higher temperature or flame is required.
5. *Autoanalyzer:* Using OCPC method.
6. *Atomic absorption spectrophotometry:* Used as a reference method

Physiology and Biochemistry of Calcium

Calcium is fifth most common element and most prevalent cation found in the body. Average human body contains approximately 1 kg of calcium.

Calcium is found in three main compartments –

Skeleton – 99 percent
Soft tissue – 1 percent
Extracellular fluid < 0.2 percent

In blood, all of the calcium is in the plasma and exists in three physiochemical states:
Free or ionized 50 percent
Bound to plasma proteins 40 percent
Complexed with small anions 10 percent
Free or ionized calcium is the biological active form. Its concentration in plasma is tightly regulated by parathyroid hormone and decreases 1,25 dihydroxy vitamin D.
Of the protein bound calcium fraction, 80% is associated with albumin, 20% with globulin.

Interpretation

Reduced Serum Calcium

- Lowest values are found in hypoparathyroidism
- Hypoalbuminemia is most common cause of reduction in concentration of total serum calcium
- Chronic liver disease
- Nephrotic syndrome
- Congestive heart failure
- Chronic renal failure
- Pseudohypoparathyroidism.

Hypercalcemia

- Primary hyperparathyroidism: Most common cause of hypercalcemia
- Malignancy with skeletal involvement
- Vitamin D overdose
- Granulomatous disease: Sarcoidosis, tuberculosis
- Renal failure: Acute, chronic.

Phosphorus

Estimation

Measurement of inorganic phosphorus in serum is done by Fisk and Subbarow method.

Principle

In acidic medium, acid molybdate reagent reacts with inorganic phosphorus to form phosphomolybdic acid and hexavalent phosphomolybdic acid is reduced by ANSA (1,2, 4-amino naphthol sulfonic acid) to give blue compound which is estimated colorimetrically.

CHAPTER 26

Amylase

Learning objectives:

1. Describe method for estimation of Serum Amylase.
2. Interpret clinical significance for Amylase estimation.

Estimation of Serum Amylase

Principle

Iodometric (or amyloclastic method):
Starch is used as a substrate and the polysaccharide remaining after a fixed time are measured by its color reaction with iodine.

Procedure

Take 25 ml starch and add 15 ml phosphate buffer, 5 ml NaCl and heat it at 90°C.
Take two test tubes and label them as test (T) and control (C).

	T (ml)	C (ml)
Starch substrate	4.5	4.5
Keep it in water bath at 37°C for 5 min.		
Serum	0.5	–
Incubate at 37° for 30 min.		
1N HCl	1	1
Serum	–	0.5

Assay

Take two 50 ml flasks and label them as T and C.

	T (ml)	C (ml)
DW	35	35
1N HCl	1	1
Solution from tube T	0.5	–
Solution from tube C	–	0.5
Working iodine	1	1

Make volume to 50 ml after mixing with distil water.
Read OD at 610 nm.

Calculations

$$\text{Serum amylase} = \frac{OD_C - OD_T}{OD_C} \times 660 \text{ U/L}$$

Other methods:
1. Saccharogenic method
2. Chromolytic method
3. Using oligosaccharide substrate
4. Turbidity

Interpretation

Amylase occurs in salivary glands, pancreas and fallopian tubes.
Pancreatic isoenzyme has highest clearance rate.

Elevated serum amylase seen in:

Acute pancreatitis
Pseudopancreatic cyst
Acute parotitis
Chronic pancreatitis
Neoplasm
Intestinal obstruction

VIVA

1. Types of amylase

2. Source of α-amylase
   a. Example

   b. Site of faction

3. α-amylase is ..... in origin
4. β-amylase is ..... amylase
5. End-products of α-amylase
6. End-products of β-amylase
7. Activator of salivary amylase
8. High serum amylase is seen most commonly in

KEY

α, β

animal

a. Salivary amylase
   Pancreatic amylase
b. Endoamylase break
   α- 1,4 glucosidic linkage

Animal
Exo-amylase
Dextrinogenic
Sac_charogenic

Cl
Pancreatitis

Questions:

1. Write the principle and procedure of estimation of serum amylase.
2. What are the causes of elevated serum amylase level?

Bibliography:

Burtis, C., Ashwood, E., Bruns, D. and Tietz, N., 2008. Tietz Fundamentals Of Clinical Chemistry. St. Louis, Mo.: Saunders Elsevier.
Kaplan, L. and Pesce, A., 2010. Clinical Chemistry. St. Louis, Mo.: Mosby/Elsevier.
Keith Wilson, John M Walker. Principles and techniques of biochemistry and molecular biology. 7th edition. Cambridge: Cambridge University Press; 2017.
Lehninger A, Nelson D, Cox M. Lehninger principles of biochemistry. New York: Worth Publishers; 2000.
Murray, R., Bender, D., Botham, K., Kennelly, P., Rodwell, V. and Weil, P., 2012. Harpers Illustrated Biochemistry 29Th Edition. Blacklick: McGraw-Hill Publishing
Tietz, N., 1987. Fundamentals Of Clinical Chemistry. Philadelphia: W.B. Saunders.
Voet, D., Voet, J. and Pratt, C., 2016. Fundamentals Of Biochemistry. New York: Wiley.

CHAPTER 27

Cholesterol

Learning objectives:

1. Explain different methods for determining total Cholesterol.
2. Discuss Applied aspects for Cholesterol estimation.
3. Describe Cholesterol metabolism and uses of cholesterol.
4. Explain drug therapy to lower Cholesterol levels.

Cholesterol

Cholesterol present in the blood occurs both as free and as cholesterol esters.

Methods for Determination of Total Cholesterol

i. *Direct method:* Utilizes serum orplasma.
ii. *Indirect method:* Involves treating sample with solvent extraction or other isolation procedure. They include: chemical, colorimetic and enzymatic methods.

Chemical Methods

Liebermann Burchard's Reaction

i. Uranyl acetate
ii. Zlatkis method

Principle

Cholesterol in acidic media reacts with acetic anhydride and sulfuric acid to form green colored complex which is read colorimetrically at 570 nm. Sulfuric acid is a dehydrating and oxidizing agent, while, iron enhances the reaction. Acetic anhydride sulfuric acid is corrosive.

Zlatkis Method

Uses acetic acid and ferric chloride.

Uranyl Acetate Method

Procedure

UA (uranyl acetate) for protein free filtrate has uranyl acetate, acetic acid, $FeCl_3$ and is known as *acetate ferric chloride precipitating (ppting) reagent*. Take three test tube and label them as test (T), standard (S) and blank (B) (Fig. 27.1).

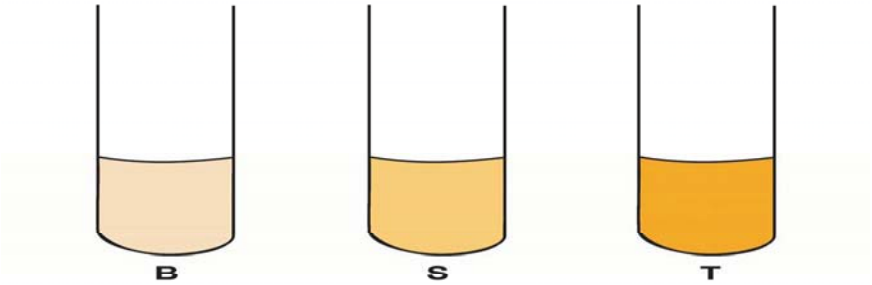

Fig. 27.1: Estimation of S. cholesterol

PFF Preparation

	T	S	B
Ppting reagent (ml)	5 (4.95)	5 (4.95)	5
Serum	50 uL	-	-
Std (2 mg/ml)	-	50 uL	-

Mix centrifuge it for 5 min

Test

	T (ml)	S (ml)	B (ml)
PFF (test)	3	-	-
PFF (std)	-	3	-
UA	-	-	3
H_2SO_4	1	1	1

Mix well, keep in dark for 10 min (red color appears)
Read at 560 nm (Fig 27.1).

Calculation

$$\text{Cholesterol} = \frac{T}{S} \times 200 \text{ mg/dL}$$

5 ml solution has 0.05 ml serum
3 ml will have $0.05/5 \times 3 = 0.03$
1 ml standard has 2 mg cholesterol
0.03 ml will have $2 \times 0.03 = 0.06$ mg/ml cholesterol
[std = 2 mg/ml = 200 mg/dL]

Precautions

1. Glass wares should be completely dry.
2. Reagents are corrosive, use dispensers.

3. $FeCl_3$—acetate should be stored at 2 to 8°C, but should be brought to room temperature before estimation.

Normal Range: Cholesterol

150 to 250 mg/dL
Conversion of mg/dL to nmol/L ÷ 38-7 or × 0.026.

Enzymatic Method

Advantage

1. More specific and sensitive
2. Linear up to 700 mg /dL
3. Low sample volume required

Applied Aspects

1. *Raised cholesterol levels (hypercholesterolemia)*
   CHD (coronary heart disease)
   Angina
   DM
   Hypothyroidism
   Obstructive jaundice
   (Because decreased excretion of bile salts increases cholesterol levels)
   NS (nephrotic syndrome)
   Hyperlipidemia (familial)
2. *Hypocholesterolemia*
   MAS (malabsorption syndrome)
   Severe wasting severe malnutrition
   Hyperthyroidism
   Hemolytic jaundice
   Anemia
   Pernicious anemia

Cholesterol Metabolism

Cholesterol is synthesized from acetyl CoA (90 percent in liver, gut) and key enzyme is HMG CoA reductase.
HMG CoA reductase enzyme is regulated by:

i. Hormone (insulin, thyroxine)
ii. Diet
iii. Feedback inhibition
iv. Synthesis

CHAPTER 28

Gastric Juice Analysis

Learning objectives:

1. Discuss composition of Gastric juice and enzymes present in it.
2. Describe Gastric juice analysis to find out its free and total acidity.
3. Explain Gastric Function tests.
4. Interpret results of Gastric juice analysis.

Gastric Juice

Composition of Gastric Juice

- Cells of gastric glands secrete 2500 ml gastric juice daily and secretion is stimulated by gastrin
- Gastric juice is produced by parietal cells and mucus is produced by mucous cells
- Gastric juice is yellow in color and consists of 95 to 98 percent water
- Normal volume is 20 to 100 ml, concentration is 155 mmol/L
- Thin light colored fluid which is acid inreaction
- Specific gravity 1.007
- pH 1.5 to 3.5
- Contains 0.5 percent solid matter

Enzymes in Gastric Juice

Digestive enzyme
Pepsinogen
Gastric lipase
Non-digestive enzymes — LDH, alkaline phosphatase ribonuclease, SGOT/SGPT, gelatinase, gastrin

Ions: NaCl, KCl, PO_4

Electrolytes : Cations : Na^+, K^+, Mg^{+2}, H^+
Anions : Cl^-, HPO_4^{2-}, SO_4^{2-}

HCl is secreted by parietal cells and HCl in gastric juice aids protein digestion.

Gastric juice has 2 functions:

i. Hydrolyzes protein present in food by activating enzyme pepsin (pepsinogen → pepsin)
ii. Bactericidal action

Mucus has 2 functions:

a. Provides moisture
b. Helps in motility of food particles

Acidity

Free acidity: It is due to free HCl present in gastric juice.
Total acidity: It is due to HCl bound to proteins. Weak organic acids like acetic acid, lactic acid, ascorbic acid, citric acid and phosphates constitute total acidity.
Acidity: It is expressed as: clinical units, mEq/l.

Clinical Unit

It is ml of 0.1 N NaOH required to neutralize 100 ml of gastric juice and this is obviously equal to ml of 0.1 N HCl.

In 100 ml of gastric juice, this corresponds numerically to concentration of acid expressed in terms of mEq/l, also known as *degree of acidity*.

It is the pH of gastric contents which determines whether or not the physiological function of gastric secretion will be served.

Indicators

Reagent/Indicator	*pH range*	*Color change*
1. Topfer's reagent gives pinkish color only in presence of free HCl	2.9 to 4.0 (3.3) gives free acidity	red-yellow intermediate color Salmon pink
2. Phenolphthalein	8.3 to 10 (9.2)	colorless – red

When gastric contents are titrated with alkali to the color change with Topfer's reagent, a measure of free HCl acid (FA) present will be obtained, value being uninfluenced by any weak acid which may be present.

If the titration is then continued to color change with phenolphthalein (pH 8.5), the total acidity (TA) is determinable.

TA – FA = combined acidity.

Need for Two Indicators

Following complete neutralization of acid (especially acetic acid) with alkali, the pH reaches 8.5 and not 7 (as expected theoretically), so we need an indicator which acts at pH 8.5 *i.e.*, phenolphthalein.

Normal Acidity

FA = 10 to 30 clinical units
Total Acidity: 30 to 50 clinical units.
Disadvantage with *Topfer's reagent:*
1. Color change not sharp
2. Color fades rapidly at end point.

Experiment

Gastric juice analysis and to find out its free and total acidity.

Procedure

Collection

i. *Introduction of stomach tube:*
In a patient kept overnight fasting
Ryle's tube is introduced after lubricating the tip through the nose.
ii. *Removal and analysis of residuum*: Because there is always some basal secretion of gastric juice, some material is present in so-called empty stomach.
iii. Discard residuum
iv. Collect sample after one hour known as BASAL SAMPLE
v. Examination of sample.

Determination of Free and Total Acidity

i. Free acidity:
Indicator – Topfer's
Fill burette with 0.1 N NaOH
Take 10 cc gastric juice in a china dish, add 2 drops of Topfer's reagent
Titrate with 0.1 N NaOH dripwise till Salmon pink.
The reading of burette at end point is measure of *free acidity*.
ii. Total acidity:
Indicator – Phenolphthalein
To the same sample go on adding 0.1 N NaOH till it turns yellow and finally a faint pink odor is obtained.
Take this burette reading. This is total measure of acidity.

Calculation

The amount of 0.1 ml N NaOH required to neutralize 10 ml of stomach content in acidity is mEq/L and multiple this reading by 10 to obtain values in clinical units.

CHAPTER 29

Spectroscopic Examination of Blood

Learning objectives:

1. Explain Spectroscopic examination of blood.
2. Describe OxyHb, CarboxyHb, MetHb in detail.

Various types of hemoglobin: Oxy Hb, carboxy Hb, Met Hb.
The term Hb implies that iron is the reduced divalent (ferrous state)

This is true whether Hb is in oxygenated form (oxy Hb) or deoxygenated form (deoxy Hb). Heme contains iron in Fe^{+2} form and tetrapyrrole ring, molecular weight of 69,000. Normally Fe^{+2} is present in heme which has capacity to bind with oxygen, carbon monoxide, *etc.* to form oxy Hb, carboxy Hb respectively. There is difference in structure of various types of Hb and they have different spectra because each Hb absorbs light at a specific wavelength and it will show a dark band and that band is characteristic of particular Hb.

The terms reduced Hb and ferro Hb which have been used as synonyms for deoxy Hb are redundant.

Oxy Hb

Oxy hemoglobin (Oxy Hb)) is formed when hemoglobin solution is shaken in air and the union is molecular.
-Iron not oxidized, remains in ferrous state
- 1g Hb combines with 1.36 ml of O2 at NTP
- Agents converting oxyHB to Hb: ammonium sulphide, sodium hydrosulphide, low oxygen pressure.

Carboxy Hb

It is Hb-CO complex.
CO (carbon mono oxide) is normally generated in small quantities from catabolism of heme, although amount of carboxy Hb formed is too small to be readily measured.

Exogenous Sources of CO

Cigarette smoking, gasoline engines, improperly ventilated home heating units.

Endogenous Sources of CO

Metabolic conversion of heme to bilirubin and accelerated in hemolytic anemia.

When inhaled, CO combines tightly with heme Fe^{+2} of Hb to form carboxy Hb. Binding affinity of Hb for CO is 250 times greater than that for O_2, so high concentration of carboxy Hb limit oxygen content of blood. Moreover, binding of CO to a Hb subunit increases O2 affinity for the remaining subunits in the Hb tetramers.

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Thus, at a given tissue pO_2 value, less O_2 dissociates from Hb when CO is also bound, shifting O_2 dissociation curve to left.

Consequently CO not only decreases O_2 content of blood, but also decreases O_2 availability to tissues, thereby producing a greater degree of tissue hypoxia than would an equivalent reduction in oxy Hb due to hypoxia alone.

CO may also bind to other heme proteins, such as myoglobin and mitochondrial cytochrome oxidase a_3, this may limit oxygen use when tissue pO_2 is very low.

The toxic effects of CO are a result of hypoxia. CO Hb although helpful in diagnosis, does not always correlate with clinical findings or prognosis.

Treatment

Removal from contaminated area, O_2 administration.

Met Hb

Acquired causes of met Hb:

1. *Drugs*: Benzocaine, chloroquine, dapsone, lignocaine, nitroglycerine, primaquine, sulfonamides
2. *Chemicals*: Nitrate, nitrate, nitrous oxide, naphthalene.

Normal concentration of met Hb < 1.5 percent of total Hb.

Congenital methemoglobinemia is due to met Hb reductase deficiency (10 to 30 percent). Met Hb > 60 percent is lethal.

Met Hb is formed by oxidation of Hb and oxy Hb e.g., by action of potassium ferrocyanide on latter and is brownish in color.

In strongly basic or strongly acidic solution, Hb is denatured to alkaline hematin or acid hematin respectively. Sulfhemoglobin, verdohemoglobin and choleglobin are degradation products of Hb.

Met Hb in plasma is result of release of heme from Hb and reassociation with plasma albumin.

Heme iron in Hb is normally in ferrous state (Fe^{+2}), when oxidized to ferric state (Fe^{+3}) met Hb is formed and this form of Hb cannot bind oxygen.

Principal physiological system to maintain Hb iron in reduced state is: NADH MetHb reductase (Diaphorase I). The NADH for this enzyme is supplied by glycolysis.

A minor pathway for met Hb reduction involves NADPH-Met Hb reductase (diaphorase II), the NADPH for this reaction comes from HMP shunt (Fig. 29.1).

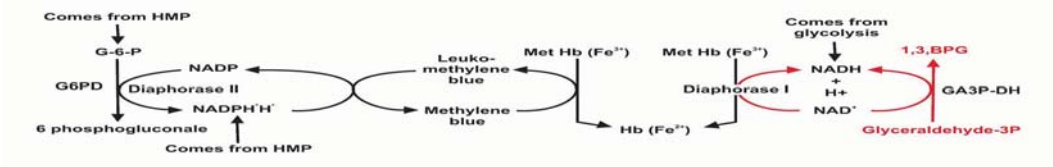

Fig. 29. 1: Minor pathway for met Hb reduction

Clinical Features

Normal value of met Hb < 1.5 percent of total Hb.
In otherwise healthy individuals:
met Hb levels up to 20 percent cause only cyanosis
20 to 50 percent cause dyspnea, weakness, syncope
50 to 70 percent - cause more severe seizures, dysarrhythmia, coma
70 percent-lethal

Hypoxia associated with decreased O_2 content of blood (and decreases O_2 dissociation from Hb) and pO_2 is normal, so, in a cyanotic patient with normal pO_2 implies possible presence of methemoglobinemia.

Treatment

Methylene blue - acts as electron-transfer between NADPH-met Hb reductase reaction (diaphorase II) and decreases activity of this system several fold.

Hb Spectroscopy

Take Hb solution in test tube and observe the bands with the help of spectroscope. Oxy Hb α-(narrow) and β band (more intense) band lie in yellow and green regions of spectrum. Normal bands in visible region have D and E dark lines (Fig. 29.2, Table 29.1):

D 581 (yellow region), E 527 (green region)

Oxy Hb show two dark bands:

α – 577 nm (yellow region)
β – 539 nm (green region)

Carboxy Hb – bands are similar to oxy Hb, but, displaced slightly (5 nm) towards violet end of spectrum (Fig. 29.2).

Action of reducing substances on bands:

Sodium dithionate (sodium hydrogen sulfite, $Na_2S_2O_4$):

Reduces met Hb but not carboxy Hb

Sulf Hb is converted to Hb and it also converts oxy Hb to Hb producing a single band in region 634, plus 2 bands of oxy Hb

Met Hb: single narrow band in red region 633 nm.

Table 29.1: Spectroscopy of different Hb

Line/Band	*Wavelength (nm)*
F-lines	589 (D) (Yellow), 529 (E) (Green)
Oxy Hb	α 577 β 539
Carboxy Hb	Similar to Oxy Hb 5 mm towards violet end
Met Hb	*634 (red) plus two bands of oxy.*

CHAPTER 30

Stone Analysis

Learning objectives:

1. Discuss procedure for Stone analysis.
2. Explain different stones.

Often a lab is requested to investigate the nature of stones concretions, or calculi that are surgically removed or excreted from the body.

Urinary tract stones are commonest and other stones include gallstones, intestinal concretions, salivary and pancreatic calculi.

Urinary Tract Stones

They arise from kidney, renal pelvis, may pass into ureter. Quantitative and qualitative analysis of chemical constituents of kidney stones is helpful in establishment of etiology, planning of therapy and sometimes distinguishing differential diagnosis. Chemical analysis of these stones confirm this sequence of events.

Characteristics: Renal Stones

- Can be simple a mixed composed of (commonest 67%)
- Calcium oxalate calcium phosphate acid (8%), uric acid (8%), cystine (1 to 2%) or a mixture of these with magnesium ammonium phosphate (struvite) (12%)
- Multiple factors are responsible for their formation urine flow, excretion of excess of insoluble substances, absence of inhibitors of precipitation of insoluble agents.

Stone Analysis: Procedure

Steps

1. Crush the given stone
2. Mix with 10 percent HCland boil on a flame
3. After cooling, distribute it to 4 test tubes.

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Tube 1

Add few drops of ammonium molybdate (5%) + 1 drop of fresh ascorbic acid solution (a pinch in 10 ml dw). Blue color if develops indicates presence of phosphates.

Tube 2

Add saturated sodium acetate solution to stone solution. White precipitate indicates presence of calcium oxalate.

Tube 3

Add 1 ml sodium carbonate (14%), 1 ml phosphotungstate to stone solution. Blue color indicates presence of uric acid.

Tube 4

During boiling if vapor of a gas comes out and extinguishes a burning stick, indicates presence of carbonate.

Common steps of analysis

- Wash with water and dry out into two halves, do gross examination, powder it
- Do flame test:
- Uric acid and cystine stone burn away completely cystines give pale blue color
  Do chemical analysis

Methods for stone analysis:
Chemicals analysis
Infrared spectroscopy
X-ray diffraction

Stone	*Appearance*	*Method*	*Inference*
Uric acid	Coloreless, hard, smooth surface	Murexide test-Add 2-3 drops conc. HNO_3 , evaporate to dryness by heating in water bath	Red or yellow residue
Calcium oxalate	Hardest, small, rough blood stained	–	–
Phosphate	–	Dissolve stone in conc. HNO_3 and add ammonium molybdate	Evolution of yellow flame
Triple	(Present in bladder) softest, whitest	–	–

Contd...

Contd...

Cystine	Small, smooth, waxy, brownish yellow or light green xanthine yellowish brown	Dissolve in conc. NH_3 an do microscopy	Murexide test negative Cystine crystals
		Dissolves in nitric acid leaving a yellow resridue.	Murexide test negative

Gallstones

Examination of gallstones is easy and substance found in them are cholesterol (commonest), bile pigments, calcium phosphate and carbonate.

Stone	*Appearance*
Cholesterol:	
Pure	Very light colored or white
Mixed	Colored, depends on type of pigment
Bile pigment	Small dark colored

Procedure

- Wash with water
- Extract with ether by boiling powdered stone in ether and ethanol.
- Carry out Liebermann Burchard reaction for cholesterol
- Add dil HCl and add molybdate, NH_3, acetic acid and ammonium oxalate to produce ppt of calcium oxalate
- Or examine chloroform extract for bilirubin with diazo reagent

Pancreatic Calculi

Usually small, rough, composed of calcium carbonate and phosphate.

Method

1. Treat with dilute HCl acid and effervescence shows presence of carbonate
2. Phosphate and oxalates when treated with nitric acid and ammonium mobybdate to give yellow ppt.

Salivary Calculi

They are composed of calcium phosphate and carbonate and analyzed in a similar way as renal stone.

Section VI

CHAPTER 31

Reference range of various parameters in Clinical Lab

Learning objectives:

1. Describe reference range of various chemical constituents of blood.

Chemical Constituents of Blood

		Range (normal)
Acetoacetate		<1 mg/dL
Acetone		<1 mg/dL
ACTH		< 120 pg/ml
Aminotransferases:		
ALT (SGPT)		10–40 U/L
AST (SGOT)		10–40 U/L
Albumin		3.5–5.2 g/dL
Amylase		60–180 U/L
Blood gases (arterial)		
	Bicarbonate	21–28 mmol/L
	pCO_2	35–45 mmHg
	pO_2	80–100 mmHg
	pH	7.38–7.44
Bilirubin		
	Total	0.2–0.8 mg/dL
	Direct	0.1–0.3 mg/dL
	Indirect	0.2–0.7 mg/dL
Calcium		
	Total	8.6–10.0 mg/dL
	Free	4.6–5.1 mg/dL
Chloride		98–106 meq/L
Cholesterol		
	Desirable	< 200mg/dL
		150–200 mg/dL
	Copper	70–140 µg/dL

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Creatine kinase		
M		15–105 U/L
F		10–80 U/L
Creatinine		0.5–1.2 mg/dL
Estrogens	F	
	Follicular	60–200 pg/ml
	Luteal	160–400 pg/ml
	Postmenopausal	<130 pg/ml
Ethanol	(mild)	50–100 mg/dL
Fat, fecal		
Infant 1–		< 1 g/d
6 yrs		< 2 g/d
Adult		< 7 g/d
Ferritin		
M		20–250 ng//ml
F		10–120 ng/ml
Fluoride		0.01–0.2 µg/ml
Folate		
Red cell		140–628 ng/ml
FSH F		
Follicular		1–10 IU/L
Follicular ovulatory peak	6–17 IU/L	
Luteal		1–9 IU/L
Postmenopausal		90–100 IU/L
M		1.4–15.4 IU/L
Glucose		
Fasting		74–106 mg/dL
Postprandial		< 140 mg/dL
GGT	M	2–30 U/L
	F	1–24 U/L
Glycated Hb	HbA_{IC}	up to 6%
GH	M	1–9 ng/ml
	F	1–16 ng/ml
	M	35–164 mg/dl
Haptoglobin	F	40–175 mg/dl
	M	
Iron	F	65–175 µg/dL
		50–170 µg/dL
LDH		90–320 U/L
LDL-C	Total	110–190 U/L
	Desirable	< 130 mg/dL

Lactate		5-15 mg/dL
Lead		
Child		< 25 µg/dL
Adult		< 25µg/dL
Lipase		31–186 U/L
LH		
M		1.24–7.8 U/L
F		
	Follicular	1.68–15.0 U/L
	Ovulatory	21.9–56.6 U/L
	Luteal	0.61–16.3 U/L
Lithium		0.6–1.2 meq/L
Magnesium		1.6–2.6 mg/dL
Mercury		0.6–5.9 µg/L
Met Hb		0.06–0.24 g/dl
Myoglobin		
	M	19–92 µg/L
	F	12–76 µg/L
Osmolality		275–295 mOsmol/kg
Phosphatase		
Acid		0–5.5 U/L
Alkaline		30–120 U/L
Phosphorus (inorganic)		2.7–4.5 mg/dl
Potassium		3.5–5.0 mmol/L
Protein		5.5–8.0 g/dl
Progesterone		
	Total	13–97 ng/dL
	M	
	F	
	Follicular	15–70 ng/dL
	Luteal	200–2500 ng/dL
Pregnancy		
7–13 wk		1025–4400 ng/dL
14–37 wk		1950–8250 ng/dL
30–42		6500–22900 ng/dL
Prolactin M		3–14.7 ng/ml
F		3.8–23.2 ng/ml
Selenium		59–234 µg/dL
Sodium		136–145 mmol/L
Specific gravity (urine)		1015–1025
Testosterone total		
	M	280–1100 ng/dL
	F	15–70 ng/ml
Thyroglobulin		3–42 ng/ml
TSH		0.32–5.0 mU/L

SUBJECT INDEX

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Simmi Kharb

Dr. Simmi Kharbcompleted her MBBS in 1989 and MD (Biochemistry) in 1995 from Pt. B. D. Sharma PGIMS, Rohtak, India. She has an extensive teaching and research experience in the field of Medical Biochemistry, Medical Biotechnology, and Clinical Chemistry, and has published 224 papers in Indexed Journals (152 International, 72 National) in the area of pathophysiology of pre-eclampsia, free radicals, diabetes, leukemia, cancer and myocardial infarction (MI). Her research on free radical biology, antioxidants, nutraceuticals, and Vitamin E & C supplementation in diabetes, MI, neonatal jaundice, leukemia and pre-eclampsia has been published in more than 30 international papers and cited in more than 300 papers. She has also worked in the area of congenital toxicology and origin of diseases, and her research on fluoride toxicity has been appreciated at several national and international scientific platforms.

Besides being a Professor in the Department of Biochemistry at Pt. B. D. Sharma PGIMS, Rohtak since 2008, she is also holding the positions of Nodal Officer for DHR-Multidisciplinary Research Unit, State Nodal Officer for AB-PMJAY Costing of Health Services in India project and Regional coordinator for Dr. Padam Singh Research and Development Scheme at the institute, and Member Secretary for Ph.D. & NAAC Assessor at Pt. B. D. Sharma UHS, Rohtak, since 2019.

She is trained in human whole genome sequencing and holds experience in supervising quality control systems in clinical chemistry labs, and is trained in microarray technology, proteomics nanotechnology, immobilized enzymes for sensors, cord blood banking, stem cell therapy, and molecular forensics. Her profile is mentioned in the "Marquis Who's Who in World" and "Marquis Who's Who in Science and Engineering". She has handled many research projects and received research grants from both central and state government bodies. She has also refereed the project for MDRS Mission (Mars Desert Research Station), NASA, USA.