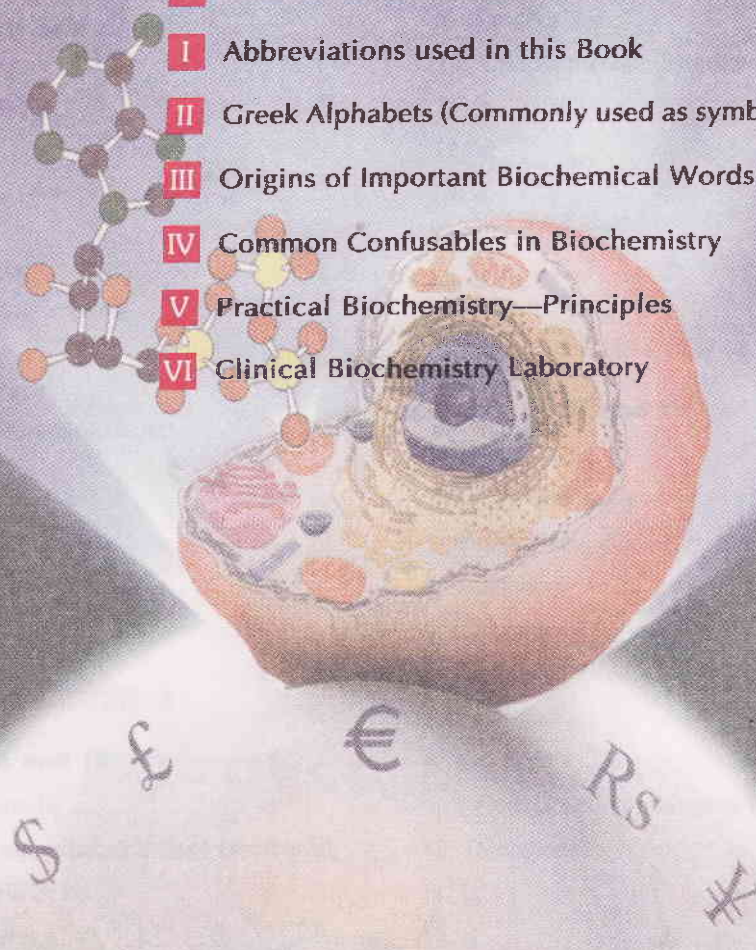


# APPENDICES

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# Answers to Self-assessment Exercises

## CHAPTER 2

### Answers to III and IV

1. Sucrose,
2. Glyceraldehyde,
3. Epimers,
4. Anomers,
5. Aglycone,
6. Streptomycin,
7.  $\alpha$ -1,6-Glycosidic bond,
8. Inulin,
9. Hyaluronic acid,
10. N-Acetylneuraminic acid,
11. b,
12. d,
13. a,
14. d,
15. a.

## CHAPTER 3

### Answers to III and IV

1. Triacylglycerols,
2. Geometric isomerism (*cis-trans* isomerism),
3. Chaulmoogric acid,
4. Triacylglycerols,
5. Stereospecific number,
6. Saponification number,
7. Dipalmitoyl lecithin,

8. Phosphatidylinositol,
9. Gangliosides,
10. Cyclopentanoperhydrophenanthrene,
11. a,
12. d,
13. d,
14. c,
15. b.

## CHAPTER 4

### Answers to III and IV

1. 16%,
2. L- $\alpha$ -Amino acids,
3. Methionine,
4. Zwitterion,
5.  $\beta$ -Alanine,
6. Peptide bonds,
7. Tryptophan,
8. 9,
9. 1-Fluoro 2,4-dinitrobenzene (FDNB),
10. Denaturation,
11. b,
12. d,
13. b,
14. d,
15. a.

**CHAPTER 5****Answers to III and IV**

1. Gene,
2. RNA,
3. Nucleotides,
4. Thymine,
5. 2,
6. Base + sugar + phosphate,
7. Erwin Chargaff,
8. 3 Hydrogen bonds (in place of 2 in A-T),
9.  $\beta$ -Form,
10. CCA(5' to 3'),
11. d,
12. b,
13. c,
14. d,
15. d.

**CHAPTER 6****Answers to III and IV**

1. In yeast,
2. Ligases,
3. Coenzyme,
4. Denaturation,
5. Alcohol dehydrogenase, carbonic anhydrase,
6. Active site,
7. NADP<sup>+</sup>,
8. E.C. 1.1.1.1,
9. AMP/ADP,
10. Creatine phosphokinase (CPK),
11. c,
12. d,
13. b,
14. d,
15. b.

**CHAPTER 7****Answers to III and IV**

1. Acetylation,
2. Riboflavin,
3. Vitamin E (tocopherol),
4. Pyridoxine (B<sub>6</sub>),
5. Avidin,
6. Pantothenic acid,
7. Cobalamin (B<sub>12</sub>),
8. Dermatitis, diarrhea and dementia,
9. Vitamin K,
10. Folic acid,
11. b,
12. d,
13. a,
14. d,
15. a.

**CHAPTER 8****Answers to III and IV**

1.  $\beta$ -Glycosidic bonds,
2. Raffinose,
3. Lactase ( $\beta$ -galactosidase),
4. Fiber,
5. Parietal (oxyntic) cells,
6. Glutathione,
7. Hartnup's disease,
8. Arginine, lysine,
9. Colipase
10. Mixed micelles,
11. a,
12. d,
13. c,
14. b,
15. a.



**CHAPTER 9****Answers to III and IV**

1. Fibrinogen,
2. Electrophoresis,
3. Hemoglobin,
4. B-Lymphocytes,
5. IgG,
6. IgE,
7. 40–50°C,
8. C-reactive protein,
9. Staurt factor (Xa),
10. Plasmin,
11. c,
12. d,
13. a,
14. b,
15. b.

**CHAPTER 10****Answers to III and IV**

1. 574,
2. Methemoglobin,
3. Carbonic anhydrase,
4. 2,3-Bisphosphoglycerate,
5. Deoxyhemoglobin,
6. Thalassemias,
7. Succinyl CoA,
8. Uroporphyrinogen synthase I,
9.  $\delta$ -Aminolevulinate synthase,
10. Biliverdin,
11. a,
12. a,
13. b,
14. d,
15. c.

**CHAPTER 11****Answers to III and IV**

1.  $\Delta G = \Delta H - T\Delta S$  (T = Absolute temperature),
2. Exergonic or spontaneous,
3. Phosphoanhydride bonds,
4. Phosphoarginine,
5. Electrons,
6. Inner mitochondrial membrane,
7. Heme (porphyrin with iron),
8. Cytochrome oxidase (cyt a + a<sub>3</sub>),
9. Cytochrome a + a<sub>3</sub>,
10. Superoxide dismutase,
11. d,
12. a,
13. b,
14. d,
15. a.

**CHAPTER 13****Answers to III and IV**

1. Thiamine, riboflavin, lipoic acid, niacin, pantothenic acid,
2. Absence of glucose 6-phosphatase,
3. L-Gulonolactone oxidase,
4. Sorbitol,
5. Galactose 1-phosphate uridyltransferase,
6. Leucine and lysine,
7. Succinate thiokinase,
8. Uronic acid pathway,
9. Glycogenin,
10. Oxaloacetate,
11. d,
12. c,
13. b,
14. a,
15. b.

**CHAPTER 14****Answers to III and IV**

1. Triacylglycerols,
2. HMG CoA reductase,
3. Ampipathic,
4. Sphingomyelinase,
5. HDL,
6. 129 ATP,
7. Zellweger syndrome,
8. Citrate,
9. HDL,
10. Unsaturated fatty acid,
11. d,
12. a,
13. d,
14. c,
15. b.

**CHAPTER 15****Answers to III and IV**

1. Pyridoxal phosphate,
2. Glutamate dehydrogenase,
3. Carbamoyl phosphate synthase I,
4. Glycine transaminase,
5. Tetrahydrobiopterin,
6. Dopamine,
7. Homogentisate,
8. Malignant carcinoid syndrome,
9. Ornithine decarboxylase,
10. Leucine,
11. b,
12. d,
13. a,
14. c,
15. a.

**CHAPTER 17****Answers to III and IV**

1. Glutamine and aspartate,
2. Allopurinol,

3. Sodium urate,
4. Xanthine oxidase,
5. Lesch-Nyhan syndrome,
6. Thioredoxin,
7. Inosine monophosphate,
8. Alloxanthine,
9. Aspartate,
10. Carbamoyl phosphate synthetase II,
11. d,
12. a,
13. c,
14. b,
15. d.

**CHAPTER 18****Answers to III and IV**

1. 9–11 mg/dl. (4.5–5.5 mEq/l.),
2. Calcitriol,
3. Phosphorus,
4. Magnesium,
5. Sodium,
6. 3.5–5.0 mEq/l,
7. Transferrin,
8. Ceruloplasmin,
9. Gusten,
10. Selenium,
11. d,
12. a,
13. b,
14. c,
15. a.

**CHAPTER 19****Answers to III and IV**

1. Adenylate cyclase,
2.  $\text{Ca}^{2+}$ ,
3. Anterior pituitary,
4. Endorphins and enkephalins,
5. Thyroperoxidase,
6. Aldosterone,
7. Vanillyl mandelic acid (VMA),

8. Dihydrotestosterone (DHT),
9. *Cholesterol*,
10. Cholecystokinin (CCK),
11. a,
12. d,
13. b,
14. c,
15. a,
12. a,
13. c,
14. b,
15. d.

## CHAPTER 20

### Answers to III and IV

1. Heme,
2. van den Bergh reaction,
3. Alanine transaminase,
4. Alkaline phosphatase,
5. Bromosulphthalein (BSP),
6. 180 mg/dl,
7. Inulin,
8. 2ml/min,
9. Ryle's tube,
10. *Pentagastrin*,
11. a,
12. d,
13. c,
14. b,
15. b.

## CHAPTER 21

### Answers to III and IV

1. Antidiuretic hormone (ADH),
2.  $\text{Na}^+$ ,
3. 285–295 milliosmoles /kg,
4. Aldosterone,
5. Carbonic acid ( $\text{H}_2\text{CO}_3$ ),
6. Bicarbonate buffer,
7. 20 : 1,
8. Ammonium ion ( $\text{NH}_4^+$ ),
9. Bicarbonate ( $\text{HCO}_3^-$ ),
10. Carbonic acid ( $\text{H}_2\text{CO}_3$ ) or  $\text{CO}_2$ ,
11. d,

## CHAPTER 22

### Answers to III and IV

1. Collagen,
2. Glycine,
3.  $\beta$ -oxalyl aminoalimine,
4. Fibrillin,
5. Glycosaminoglycans,
6. Sarcomere,
7. Actin,
8. Calcium caseinate,
9. Lecithin/Sphingomyelin,
10. Vitamin C,
11. c,
12. d,
13. a,
14. c,
15. b.

## CHAPTER 23

### Answers to III and IV

1. 4.128,
2. Thyroid gland,
3. Fiber,
4. Carbohydrates,
5. Chemical score,
6. 1g/kg body weight/day,
7. Biological value (BV) of protein,
8. Sulfur containing amino acids,
9. Iron,
10. Plasma albumin,
11. a,
12. d,
13. c,
14. d,
15. a.

**CHAPTER 24****Answers to III and IV**

1. DNA helicase,
2. Okazaki pieces,
3. DNA polymerase III,
4. DNA topoisomerases,
5. Cyclins,
6. Telomere,
7. Transposons or transposable elements,
8. Mutation,
9. Missense,
10. Hereditary nonpolyposis colon cancer,
11. c,
12. a,
13. b,
14. a,
15. b.

**CHAPTER 25****Answers to III and IV**

1. Genome,
2. hnRNA,
3. Introns,
4. Reverse transcriptase,
5. Wobble hypothesis,
6. Ribosomes,
7. rRNA,
8. Chaperones,
9. Prion diseases,
10. Protein targeting,
11. d,
12. c,

13. a,

14. b,

15. a.

**CHAPTER 26****Answers to III and IV**

1. 30,000–40,000,
2. Constitutive genes,
3. One cistron-one subunit concept,
4. Protein-DNA complex,
5. a,
6. b,
7. a,
8. d.

**CHAPTER 27****Answers to III and IV**

1. *Escherichia coli*,
2. RNA,
3. Dot-blotting,
4. *Thermus aquaticus*,
5. Genomic library/DNA library,
6. Site-directed mutagenesis,
7. Humulin,
8. Hepatitis B vaccine,
9. Mouse,
10. Sheep (Dolly),
11. c,
12. d,
13. d,
14. a,
15. c.



# Appendix I : Abbreviations used in this Book

|          |   |                 |  |
|----------|---|-----------------|--|
| A        | <i>adenine, adenosine</i>                     | BPG             | <i>bisphosphoglycerate (2,3-BPG, 1,3-BPG)</i>            |
| Ab       | <i>antibody</i>                               | BSP             | <i>bromosulphthalein</i>                                 |
| ACP      | <i>acyl carrier protein</i>                   | BUN             | <i>blood urea nitrogen</i>                               |
| ACTH     | <i>adrenocorticotrophic hormone</i>           | BV              | <i>biological value</i>                                  |
| Acyl CoA | <i>fatty acid derivative of coenzyme A</i>    | C               | <i>cytosine, cytidine</i>                                |
| ADA      | <i>adenosine deaminase</i>                    | CA              | <i>carbonic anhydrase</i>                                |
| ADH      | <i>alcohol dehydrogenase</i>                  | Cal             | <i>calorie</i>   |
| ADH      | <i>antidiuretic hormone</i>                   | Cam             | <i>calmodulin</i>  |
| ADP      | <i>adenosine diphosphate</i>                  | cAMP            | <i>3',5'-cyclic adenosine monophosphate (cyclic AMP)</i> |
| AFP      | <i>α-fetoprotein</i>                          | CAP             | <i>catabolite activator protein</i>                      |
| AFLP     | <i>amplified fragment length polymorphism</i> | CBG             | <i>corticosteroid binding globulin</i>                   |
| Ag       | <i>antigen</i>                                | CCK             | <i>cholecystokinin</i>                                   |
| A/G      | <i>albumin/globulin (ratio)</i>               | cDNA            | <i>complementary DNA</i>                                 |
| AIDS     | <i>acquired immunodeficiency syndrome</i>     | CD <sub>4</sub> | <i>cluster determinant antigen 4</i>                     |
| ALA      | <i>δ-aminolevulinic acid</i>                  | CDP             | <i>cytidine diphosphate</i>                              |
| ALP      | <i>alkaline phosphatase</i>                   | CEA             | <i>carcinoembryonic antigen</i>                          |
| ALT      | <i>alanine transaminase</i>                   | CF              | <i>cystic fibrosis</i>                                   |
| AMP      | <i>adenosine monophosphate</i>                | CFTR            | <i>cystic fibrosis transmembrane regulator</i>           |
| APC      | <i>antigen presenting cell</i>                | cGMP            | <i>3',5'-cyclic guanosine monophosphate</i>              |
| Apo-A    | <i>apoprotein-A</i>                           | C <sub>H</sub>  | <i>constant heavy chain</i>                              |
| AP sites | <i>apurinic sites</i>                         | CHD             | <i>coronary heart disease</i>                            |
| AST      | <i>aspartate transaminase</i>                 | ChE             | <i>cholinesterase</i>                                    |
| AT       | <i>α<sub>1</sub>-antitrypsin</i>              | Chl             | <i>chlorophyll</i>                                       |
| ATCase   | <i>aspartate transcarbamoylase</i>            | C <sub>L</sub>  | <i>constant light chain</i>                              |
| ATP      | <i>adenosine triphosphate</i>                 | CLIP            | <i>corticotropin like intermediate lobe peptide</i>      |
| BAL      | <i>British antilewisite</i>                   | CMP             | <i>cytidine monophosphate</i>                            |
| BAO      | <i>basal acid output</i>                      | CNS             | <i>central nervous system</i>                            |
| BHA      | <i>butylated hydroxyanisole</i>               | CoA or CoASH    | <i>coenzyme A</i>  |
| BHT      | <i>butylated hydroxy toluene</i>              | COHb            | <i>carboxyhemoglobin</i>                                 |
| BMR      | <i>basal metabolic rate</i>                   |                 |  |
| BOAA     | <i>β-oxalylaminoalanine</i>                   |                 |  |
| bp       | <i>base pair</i>                              |                 |  |
| BP       | <i>blood pressure</i>                         |                 |  |



|                |  |                   |                                   |
|----------------|--|-------------------|-----------------------------------|
| COMT           | catechol-o-methyltransferase                       | ELISA             | enzyme-linked immunosorbent assay |
| CoQ            | coenzyme Q (ubiquinone)                            | EM                | Embden-Meyerhof                   |
| CPK (CK)       | creatine phosphokinase (creatine kinase)           | ER                | endoplasmic reticulum             |
| CPPP           | cyclopentanoperhydrophenanthrene                   | ES                | enzyme-substrate complex          |
| CPS            | carbamoyl phosphate synthase                       | ES cells          | embryonic stem cells              |
| CRH            | corticotropin releasing hormone                    | E-site            | exist site                        |
| CS             | chorionic somatomammotropin                        | ETC               | electron transport chain          |
| CSF            | cerebrospinal fluid                                | FA                | fatty acid                        |
| CT             | calcitonin   | Fab               | antigen binding fragment          |
| CTP            | cytidine triphosphate                              | FAD               | flavin adenine dinucleotide       |
| dA             | deoxyadenosine                                     | FADH <sub>2</sub> | reduced FAD                       |
| dADP           | deoxyadenosine diphosphate                         | FAS               | fatty acid synthase               |
| DG             | diacylglycerol                                     | F 1, 6-BP         | fructose 1, 6-bisphosphate        |
| DAM            | diacetyl monoxime                                  | F 2, 6-BP         | fructose 2, 6-bisphosphate        |
| dAMP           | deoxyadenosine monophosphate                       | Fc                | crystalline fragment              |
| dATP           | deoxyadenosine triphosphate                        | FDNB              | 1-fluoro 2, 4-dinitrobenzene      |
| dCMP           | deoxycytidine monophosphate                        | FFA               | free fatty acid                   |
| DCT            | distal convoluted tubule                           | FGF               | fibroblast growth factor          |
| DEAE           | diethyl aminoethylamine                            | FH <sub>4</sub>   | tetrahydrofolate                  |
| DFP or DIFP    | diisopropyl fluorophosphate                        | FIGLU             | formiminoglutamic acid            |
| dGMP           | deoxyguanosine monophosphate                       | fMet              | N-formylmethionine                |
| DHAP           | dihydroxyacetone phosphate                         | FMN               | flavin mononucleotide             |
| DHCC           | dihydroxycholecalciferol (1, 25-DHCC; 24, 25-DHCC) | FMNH <sub>2</sub> | reduced FMN                       |
| DHEA           | dehydroepiandrosterone                             | F 1-P             | fructose 1-phosphate              |
| DHF            | dihydrofolate                                      | F 6-P             | fructose 6-phosphate              |
| DHT            | dihydrotestosterone                                | Fp                | flavoprotein                      |
| DIT            | diiodotyrosine                                     | FSH               | follicle stimulating hormone      |
| dl             | deciliter  | FTM               | fractional test meal              |
| DMB            | dimethyl benzimidazole                             | G                 | guanine, guanosine                |
| DMS            | dimethyl sulfate                                   | g                 | gram                              |
| DNA            | deoxyribonucleic acid                              | ΔG                | free energy change                |
| DNase          | deoxyribonuclease                                  | GABA              | γ-aminobutyric acid               |
| DNP            | 2, 4-dinitrophenol                                 | GAG               | glycosaminoglycans                |
| DOPA           | dihydroxy phenylalanine                            | Gal-Cer           | galactocerebroside                |
| DPG            | diphosphoglycerate                                 | GAR               | glycinamide ribotide              |
| DPP            | dimethyl allyl pyrophosphate                       | GDH               | glutamate dehydrogenase           |
| dTMP           | deoxythymidine monophosphate                       | GDP               | guanosine diphosphate             |
| E <sub>o</sub> | redox potential                                    | GFR               | glomerular filtration rate        |
| EC             | enzyme commission                                  | GGT (GT)          | γ-glutamyl transpeptidase         |
| ECF            | extracellular fluid                                | GH                | growth hormone                    |
| EDRF           | endothelium-derived releasing factor               | GHRH              | growth hormone releasing hormone  |
| EDTA           | ethylene diamine tetraacetate                      | GIP               | gastric inhibitory peptide        |
| EF             | elongation factor                                  | GIT               | gastrointestinal tract            |
| EFA            | essential fatty acids                              | Gla               | γ-carboxy glutamate               |
| eIFs           | eukaryotic initiation factors                      | GLC               | gas liquid chromatography         |
| EGF            | epidermal growth factor                            | Glu-Cer           | glucocerebroside                  |
|                |  | Gly               | glycine                           |
|                |  | GLUT              | glucose transporters              |

|                   |  |                                      |   |
|-------------------|--|--------------------------------------|---|
| GN                | glucose-nitrogen (ratio)                       | Ig                                   | immunoglobulin                          |
| GMP               | guanosine monophosphate                        | IgG                                  | immunoglobulin G                        |
| GnRH              | gonadotropin releasing hormone                 | IGF                                  | insulin-like growth factor              |
| GRH               | growth hormone releasing hormone               | IL                                   | interleukins                            |
| GRIH              | growth hormone release-inhibiting hormone      | IMP                                  | inosine monophosphate                   |
| G 6-P             | glucose 6-phosphate                            | INH                                  | isonicotinic acid hydrazide (isoniazid) |
| G 6-PD            | glucose 6-phosphate dehydrogenase              | InsP <sub>2</sub> (IP <sub>2</sub> ) | inositol 1, 4-bisphosphate              |
| GPP               | geranyl pyrophosphate                          | InsP <sub>3</sub> (IP <sub>3</sub> ) | inositol 1, 4, 5-triphosphate           |
| GSH               | glutathione (reduced form)                     | IPP                                  | isopentenyl pyrophosphate               |
| GSSG              | glutathione (oxidized form)                    | IR                                   | infrared                                |
| GTP               | guanosine triphosphate                         | ITP                                  | inosine triphosphate                    |
| GTT               | glucose tolerance test                         | IU                                   | international unit                      |
| ΔH                | change in enthalpy                             | IV                                   | intravenous                             |
| HAC               | human artificial chromosome                    | K                                    | dissociation constant                   |
| Hb                | hemoglobin                                     | KA                                   | King Armstrong                          |
| HbA <sub>1</sub>  | adult hemoglobin                               | K <sub>a</sub>                       | dissociation constant of acid           |
| HbA <sub>1C</sub> | glycosylated hemoglobin                        | Kbp                                  | kilo base pair                          |
| HbF               | fetal hemoglobin                               | KD                                   | kilodalton                              |
| HbO <sub>2</sub>  | oxyhemoglobin                                  | Keq                                  | equilibrium constant                    |
| HBsAg             | hepatitis B surface antigen                    | α-KG                                 | α-ketoglutarate                         |
| HbS               | sickle-cell hemoglobin                         | Ki                                   | inhibition constant                     |
| hCG               | human chorionic gonadotropin                   | K <sub>m</sub>                       | Michaelis constant                      |
| HDL               | high density lipoproteins                      | KJ                                   | kilojoule                               |
| HGPRT             | hypoxanthine guanine phosphoribosyltransferase | LATS                                 | long acting thyroid stimulator          |
| HIAA              | hydroxy indole acetic acid                     | LCAT                                 | lecithin cholesterol acyltransferase    |
| HIF               | Hypoxia inducible transcription factor         | LDH                                  | lactate dehydrogenase                   |
| HIV               | human immunodeficiency virus                   | LDL                                  | low density lipoproteins                |
| HLA               | human leukocyte antigen                        | LFT                                  | liver function tests                    |
| HLH               | helix-loop-helix                               | LH                                   | luteinizing hormone                     |
| HMG CoA           | β-hydroxy β-methylglutaryl CoA                 | LINES                                | Long interspersed elements              |
| HMP               | hexose monophosphate                           | LPH                                  | lipotropic hormone (lipotropin)         |
| HNPPC             | hereditary nonpolyposis colon cancer           | LT                                   | leukotrienes                            |
| hnRNA             | heterogeneous nuclear RNA                      | Lp-a                                 | lipoprotein-a                           |
| Hp                | haptoglobin                                    | LSD                                  | lysergic acid diethylamide              |
| HPLC              | high performance liquid chromatography         | M                                    | molar                                   |
| HRE               | hormone responsive element                     | MAO                                  | maximal acid output                     |
| Hsp               | heat shock protein                             | MAO                                  | monoamine oxidase                       |
| 5HT               | 5-hydroxytryptamine                            | Mb                                   | myoglobin                               |
| HTH               | helix-turn-helix                               | MbO <sub>2</sub>                     | oxymyoglobin                            |
| ICD               | isocitrate dehydrogenase                       | MCAD                                 | medium chain acyl CoA dehydrogenase     |
| IDDM              | insulin dependent diabetes mellitus            | MDH                                  | malate dehydrogenase                    |
| IDL               | intermediate density lipoproteins              | mEq                                  | milliequivalents                        |
| IDP               | inosine diphosphate                            | mg                                   | milligram                               |
| IF                | initiation factor                              | MHC                                  | major histocompatibility complex        |
|                   |  | MI                                   | myocardial infarction                   |
|                   |  | MIT                                  | monoiodotyrosine                        |
|                   |  | mol                                  | mole(s)                                 |



|                   |   |                  |  |
|-------------------|---|------------------|--|
| mM                | millimolar                                  | PFK              | phosphofructokinase                      |
| mol. wt.          | molecular weight                            | PG               | prostaglandins                           |
| mRNA              | messenger RNA                               | PGA              | pteroyl glutamic acid                    |
| MSH               | melanocyte stimulating hormone              | pH               | negative log of (H <sup>+</sup> )        |
| mtDNA             | mitochondrial DNA                           | PI               | phosphatidyl inositol                    |
| MW                | molecular weight                            | Pi               | inorganic phosphate                      |
| NAD <sup>+</sup>  | nicotinamide adenine dinucleotide           | p <sup>I</sup>   | isoelectric pH                           |
| NADH              | reduced NAD <sup>+</sup>                    | PIF              | prolactin inhibitory factor              |
| NADP <sup>+</sup> | nicotinamide adenine dinucleotide phosphate | PIP <sub>2</sub> | inositol 4, 5-bisphosphate               |
| NADPH             | reduced NADP <sup>+</sup>                   | p <sup>Ka</sup>  | negative log of K <sub>a</sub>           |
| NAG               | N-acetylglutamate                           | PKU              | phenylketonuria                          |
| NANA              | N-acetylneuraminic acid                     | PL               | phospholipid                             |
| NDP               | nucleoside diphosphate                      | PLP              | pyridoxal phosphate                      |
| NE                | niacin equivalents                          | pO <sub>2</sub>  | partial pressure of O <sub>2</sub>       |
| NEFA              | non esterified fatty acid                   | POMC             | pro-opiomelanocortin                     |
| ng                | nanogram (10 <sup>-9</sup> g)               | PPi              | inorganic pyrophosphate                  |
| NGF               | nerve growth factor                         | ppm              | parts per million                        |
| NIDDM             | non-insulin dependent diabetes mellitus     | PRIH             | prolactin release-inhibiting hormone     |
| NMP               | nucleoside monophosphate                    | PRL              | prolactin                                |
| NMR               | nuclear magnetic resonance                  | PRPP             | 5-phosphoribosyl 1-pyrophosphate         |
| NPN               | non-protein nitrogen                        | PT               | prothrombin time                         |
| NPU               | net protein utilization                     | PTH              | parathyroid hormone                      |
| OAA               | oxaloacetate                                | PTH              | phenyl thiohydantoin                     |
| Ob                | obese                                       | PUFA             | polyunsaturated fatty acids              |
| OD                | optical density                             | QPRT             | quinolinate phosphoribosyltransferase    |
| OMP               | orotidine monophosphate                     | RACE             | rapid amplification of cDNA ends         |
| Osm               | osmoles                                     | RAIU             | radioactive iodine uptake                |
| PABA              | para amino benzoic acid                     | RAPD             | random amplified polymorphic DNA         |
| PAF               | platelet-activating factor                  | ras              | rat sarcoma                              |
| PAGE              | polyacrylamide gel electrophoresis          | RBC              | red blood cells                          |
| PAH               | para amino hippurate                        | RBP              | retinol binding protein                  |
| PAPS              | phosphoadenosine phosphosulfate             | RDA              | recommended dietary (daily) allowance    |
| PBG               | porphobilinogen                             | rDNA             | recombinant DNA                          |
| PBI               | protein bound iodine                        | RE               | retinol equivalents                      |
| PCM               | protein-calorie malnutrition                | RER              | rough endoplasmic reticulum              |
| PCNA              | proliferating cell nuclear antigen          | RF               | releasing factor                         |
| pCO <sub>2</sub>  | partial presence of CO <sub>2</sub>         | Rf               | ratio of fronts                          |
| PCR               | polymerase chain reaction                   | RFC              | replication factor C                     |
| PCT               | proximal convoluted tubule                  | RFLP             | restriction fragment length polymorphism |
| PDGF              | platelet derived growth factor              | R-form           | relaxed form                             |
| PDH               | pyruvate dehydrogenase                      | RIA              | radioimmunoassay                         |
| PEG               | polyethylene glycol                         | RMR              | resting metabolic rate                   |
| PEM               | protein-energy malnutrition                 | RNA              | ribonucleic acid                         |
| PEP               | phosphoenol pyruvate                        | RNAP             | RNA polymerase                           |
| PER               | protein efficiency ratio                    | RNase            | ribonuclease                             |
| PEST              | proline, glutamine, serine, threonine       |                  |  |



|                 |  |                  |                                 |
|-----------------|--|------------------|---------------------------------|
| R 5-P           | ribose 5-phosphate                           | TGF              | transforming growth factor      |
| RPA             | replication protein A                        | THF              | tetrahydrofolate                |
| RQ              | respiratory quotient                         | TIBC             | total iron binding capacity     |
| rRNA            | ribosomal RNA                                | TLC              | thin layer chromatography       |
| RSV             | rouse sarcoma virus                          | T <sub>m</sub>   | tubular maximum                 |
| RT              | reverse transcriptase                        | TMP              | thymidine monophosphate         |
| rT <sub>3</sub> | reverse T <sub>3</sub>                       | TNF              | tumor necrosis factor           |
| SAM             | S-adenosylmethionine                         | tPA              | tissue plasminogen activator    |
| SCID            | severe combined immunodeficiency             | TPP              | thiamine pyrophosphate          |
| SDA             | specific dynamic action                      | TRH              | thyrotropin releasing hormone   |
| Sf              | Svedberg floatation                          | tRNA             | transfer RNA                    |
| SGOT            | serum glutamate oxaloacetate<br>transaminase | TSH              | thyroid stimulating hormone     |
| SGPT            | serum glutamate pyruvate<br>transaminase     | TX               | thromboxane                     |
| SHBG            | sex hormone binding globulin                 | μm               | micrometer (10 <sup>-6</sup> m) |
| SIDS            | sudden infant death syndrome                 | UBG              | urobilinogen                    |
| SINEs           | short interspersed elements                  | UCP              | uncoupling protein              |
| sn              | stereospecific number                        | UDP              | uridine diphosphate             |
| SNPs            | single nucleotide polymorphisms              | UDPG             | uridine diphosphate glucose     |
| snRNA           | small nuclear RNA                            | μl               | microliter (10 <sup>-6</sup> l) |
| snRNP           | small nuclear ribonucleoprotein              | μM               | micromoles (10 <sup>-6</sup> M) |
| sRNA            | soluble RNA                                  | UMP              | uridine monophosphate           |
| SRS             | slow reacting substance                      | UTP              | uridine triphosphate            |
| STRs            | simple tandem repeats                        | UV               | ultraviolet                     |
| T               | thymine, thymidine                           | V <sub>H</sub>   | variable heavy chain            |
| T               | thymus (T-lymphocyte)                        | VIP              | vasoactive intestinal peptide   |
| T <sub>3</sub>  | 3,5,3'-triiodothyronine                      | V <sub>L</sub>   | variable light chain            |
| T <sub>4</sub>  | 3,5,3',5'-tetraiodothyronine<br>(thyroxine)  | VLDL             | very low density lipoproteins   |
| TBG             | thyroxine binding globulin                   | VMA              | vanillyl mandelic acid          |
| TBPA            | thyroxine binding prealbumin                 | V <sub>max</sub> | velocity maximum                |
| TCA             | tricarboxylic acid                           | VNTRs            | variable number tandem repeats  |
| TF              | tissue factor                                | WBC              | white blood cells               |
| T-form          | taut or tense form                           | XMP              | xanthosine monophosphate        |
| TG              | triacylglycerol                              | XP               | xeroderma pigmentosum           |
| Tgb             | thyroglobulin                                | Xyl              | xylose                          |
|                 |  | YAC              | yeast artificial chromosome     |

## Appendix II : Greek Alphabets (Commonly used as symbols)

| Alphabet | Symbol     |
|----------|------------|
| Alpha    | $\alpha$   |
| Beta     | $\beta$    |
| Gamma    | $\gamma$   |
| Delta    | $\delta$   |
| Epsilon  | $\epsilon$ |
| Zeta     | $\zeta$    |
| Eta      | $\eta$     |
| Theta    | $\theta$   |
| Kappa    | $\kappa$   |
| Lambda   | $\lambda$  |
| Mu       | $\mu$      |
| Xi       | $\xi$      |
| Pi       | $\pi$      |
| Rho      | $\rho$     |
| Sigma    | $\sigma$   |
| Phi      | $\phi$     |
| Chi      | $\chi$     |
| Psi      | $\psi$     |
| Omega    | $\omega$   |

## Appendix III : Origins of Important Biochemical Words

- Acid** (*Latin*) acidus–sour
- Acidosis** (*Latin*) acidus–sour; osis–condition
- Albinism** (*Greek*) albino–white
- Alkali** (*Arabic*) al-qite–ashes of saltwort
- Allergy** (*Greek*) allos–other; ergon–work
- Alloseric** (*Greek*) allo–the other
- Amentia** (*Latin*) amentis–mental deficiency
- Amnesia** (*Greek*) a–not; mnesis–memory
- Amphipathic** (*Greek*) amphi–both; pathos–feeling
- Amphiphilic** (*Greek*) amphi–both; philic–love
- Anaerobe** (*Greek*) a–not; aer–air; bios–life
- Anaplerotic** (*Greek*) ana–up; plerotikos–to fill
- Androgen** (*Greek*) aner–man; genesis–production
- Anemia** (*Greek*) a–not; haima–blood
- Anorexia** (*Greek*) a–not; orexis–appetite
- Anticoagulant** anti (*Greek*)–against; coagulare (*Latin*)–to curdle
- Antimetabolite** (*Greek*) anti–against; metabole–change
- Arteriosclerosis** arteria (*Latin*)–artery; sclerosis (*Greek*) hardening.
- Arthritis** (*Greek*) arthron–joint; itis–inflammation
- Atherosclerosis** (*Greek*) athere–porridge; sclerosis–hardening
- Beri-beri** (*Singhalese*)–I cannot (said twice)
- Biochemistry** (*Greek*) bios–life; chymos–juice
- Biology** (*Greek*) bios–life; logos–discourse
- Bovine** (*Latin*) bovinus–pertaining to cow or ox
- Calorie** (*Latin*) calor–heat
- Cancer** (*Latin*) crab
- Carbohydrate** carbo (*Latin*)–coal; hydor (*Greek*)–water
- Caries** (*Latin*)–decay
- Casein** (*Latin*) caseus–cheese
- Catabolism** (*Greek*) kata–down; ballein–to throw
- Catalysis** (*Greek*) kata–down; lysis–degradation
- Cathepsin** (*Greek*) to digest
- Cephalins** (*Greek*) kephale–head
- Cheilitis** (*Greek*) cheilos–lip; itis–inflammation
- Cheilosis** (*Greek*) cheilos–lip; osis–condition
- Chirality** (*Greek*) cheir–hand
- Chlorophyll** (*Greek*) chloros–pale green; phyllon–leaf
- Cholelithiasis** (*Greek*) chole–bile; lithos–stone; asis–condition
- Cholesterol** (*Greek*) chole–bile; sterol–solid alcohol
- Chromatography** (*Greek*) chroma–colour; graphein–to write
- Chromosome** (*Greek*) chroma–colour; soma–body
- Chyle** (*Greek*) chylos–juice
- Chyluria** (*Greek*) chylos–juice; auron–urine
- Chyme** (*Greek*) chymos–juice



- Cirrhosis** (*Greek*) kirrhos—orange-tawny; osis—condition
- Cis** (*Latin*) same side
- Coagulation** (*Greek*) coagulare—to curdle
- Collagen** (*Greek*) kolla—glue; genesthai—to be produced
- Colloid** (*Greek*) kolla—glue; eidos—form
- Consanguinity** (*Latin*) con—with; sanguis—blood
- Creatine** (*Greek*) kreas—flesh
- Cristae** (*Latin*) crests
- Cutaneous** (*Latin*) cutis—skin
- Cytology** (*Greek*) kytos—cell; logos—discourse
- Cytoplasm** (*Greek*) kytos—cell; plassein—to mould
- Dermatitis** (*Greek*) derma—skin; itis—inflammation
- Diabetes mellitus** (*Greek*) diabetes—running through (or a siphon); mellitus—sweet
- Eicosanoids** (*Greek*) eikosi—twenty
- Embolism** (*Greek*) embolos—to plug
- Emphysema** (*Greek*) emphysan—to inflate
- Enkephalin** (*Greek*) in the brain
- Enthalpy** (*Greek*) to warm within
- Entropy** (*Greek*) in turning
- Enzyme** (*Greek*) in yeast
- Erythrocyte** (*Greek*) erythros—red; kytos—cell
- Eukaryotes** (*Greek*) eu—true; karyon—nucleus
- Ferrous** (*Latin*) ferrum—iron
- Folate** (*Latin*) folium—leaf
- Galactose** (*Greek*) gala—milk
- Gastritis** (*Greek*) gaster—belly; itis—inflammation
- Gene** (*Greek*) genesis—descent
- Genome** (*Greek*) genos—birth
- Globin** (*Latin*) globus—ball
- Globulin** (*Latin*) globulus—little ball
- Glossitis** (*Greek*) glossa—tongue; itis—inflammation
- Glycolysis** (*Greek*) glycos—sweet; lysis—dissolution
- Goitre** (*Latin*) gultur—throat
- Gonadotrophin** (*Greek*) gona—generation; trophe—nourishment
- Hemoglobin** haima (*Greek*)—blood; globus (*Latin*)—ball
- Hepatitis** (*Greek*) hepar—liver; itis—inflammation
- Hormone** (*Greek*) hormain—to excite
- Hydrophilic** (*Greek*) hydro—water; philic—living
- Hydrophobic** (*Greek*) hydro—water; phobic—hating
- Hyperglycemia** (*Greek*) hyper—above; glycos—sweet; haima—blood
- Hypertonic** (*Greek*) hyper—above; tonos—tension
- Hypoglycemia** (*Greek*) hypo—below; glycos—sweet; haima—blood
- Hypotonic** (*Greek*) hypo—below; tonos—tension
- Icterus** (*Greek*) ikteros—jaundice
- Immunity** (*Latin*) immunis—exempt from public burden
- Inflammation** (*Latin*) inflammare—to set on fire
- In situ** (*Latin*) in the correct position
- In vitro** (*Latin*) in a test tube
- In vivo** (*Latin*) in the living tissue
- Isomerism** (*Greek*) iso—equal; mesos—part
- Isotonic** (*Greek*) iso—equal; tonos—tension
- Isotope** (*Greek*) iso—equal; topos—place
- Jaundice** (*French*) jaune—yellow
- Keratin** (*Greek*) keras—horn
- Kwashiorkor** (*Ga-African*) sickness of the deposed child
- Lactalbumin** (*Greek*) lac—milk; albumin—white
- Lecithin** (*Greek*) lekithos—egg yolk
- Lipids** (*Greek*) lipos—fat
- Lactosuria** lac (*Latin*)—milk; ovron (*Greek*)—urine
- Leukocytes** (*Greek*) leukos—white; kytos—cell
- Leukoderma** (*Greek*) leukos—white; derma—skin
- Ligase** (*Greek*) ligate—to bind
- Malaria** (*Italian*) bad air
- Malnutrition** (*Latin*) malus—bad; nutrire—nourishment
- Marasmus** (*Greek*) to waste
- Melanin** (*Greek*) melan—black
- Menopause** (*Greek*) men—month; pausis—stopping
- Metabolism** (*Greek*) metabole—change

- Mitochondria** (*Greek*) mitos–thread; chondros–granule
- Mitosis** (*Greek*) mitos–thread; osis–condition
- Monosaccharide** (*Greek*)–mono–one; saccharin–sugar
- Myeloma** (*Greek*) myelos–marrow; oma–tumor
- Nephritis** (*Greek*) nephros–kidney; itis–inflammation
- Neurosis** (*Greek*) neuron–nerve; osis–condition
- Oedema or edema** (*Greek*) oidema–swelling
- Oligosaccharides** (*Greek*) oligo–few; saccharon–sugar
- Osmosis** (*Greek*)–push
- Osteomalacia** (*Greek*) osteon–bone; malakia–softness
- Oxyntic** (*Greek*) oxynein–to make acid
- Oxytocin** (*Greek*)–rapid birth
- Palindrome** (*Greek*)–to run back again
- Pantothenic acid** (*Greek*) pantos–everywhere
- Pathogenesis** (*Greek*) pathos–disease; genesis–producing
- Pellagra** (*Italian*)–rough skin
- Pepsin** (*Greek*) pepsis–digestion
- Phagocytosis** (*Greek*) phagein–to eat; kytos–cell; osis–condition
- Phobia** (*Greek*) phobos–fear
- Polysaccharide** (*Greek*) poly–many; saccharin–sugar
- Porphyrin** (*Greek*) porphyra–purple colour
- Post-prandial** (*Latin*)–after food
- Prokaryotes** (*Greek*) pro–before; karyon–nucleus
- Proteins** (*Greek*) proteios–holding first place
- Rickets** (*Old English*) wrickken–to twist
- Serum** (*Latin*)–whey
- Sphingosine** (*Greek*) sphingein–to bind tight
- Steatorrhea** (*Greek*) stear–fat; rheein–to flow
- Stereoisomerism** (*Greek*) stero–space
- Sterol** (*Greek*) steros–solid; ol–alcohol
- Thalassemia** (*Greek*) thalassa–sea
- Thermodynamics** (*Greek*) therme–heat; dynamics–power
- Thermogenesis** (*Greek*) therme–heat; genesis–production
- Thrombosis** (*Greek*) thrombos–clot; osis–condition
- Thylakoid** (*Greek*) thylakos–a sac or pouch
- Tocopherol** (*Greek*) tokos–child birth; pheros–to bear; ol–alcohol
- Trans** (*Latin*) across
- Tumor** (*Latin*) swelling
- Vitamin** (coined inappropriately in 1906) (*Latin*) vita–life; amine
- Xanthoma** (*Greek*) xanthos–yellow
- Xenobiotics** (*Greek*) xenos–strange
- Zwitterion** (*German*) zwitter–hybrid.



## Appendix IV : Common Confusables in Biochemistry

**Acetone; acetate** – Acetone is a ketone; acetate is a carboxylic acid.

**Acetyl CoA; acyl CoA** – Acetyl CoA is a specific compound containing acetate bound to coenzyme A; acyl CoA is a general term used to refer to any fatty acid (acyl group) bound to coenzyme A.

**Albumin; albinism** – Albumin is a serum protein; albinism is a genetic disease in tyrosine metabolism.

**Amino; imino** – Amino group ( $-\text{NH}_2$ ) is found in majority of amino acids; imino group ( $=\text{NH}$ ) is present in a few amino acids like proline and hydroxyproline.

**Anabolism; catabolism** – Anabolism refers to the biosynthetic reactions involving the formation of complex molecules from simpler ones; catabolism is concerned with the degradation of complex molecules to simpler ones with a concomitant release of energy.

**Anomers; epimers** – Anomers refer to two stereoisomers of a sugar that differ in configuration around a single carbonyl atom; epimers are two stereoisomers that differ in configuration around one asymmetric carbon of a sugar possessing two or more asymmetric carbon atoms.

**Apoenzyme; coenzyme** – Apoenzyme is the protein part of the functional enzyme (holoenzyme); coenzyme is the non-protein organic part associated with enzyme activity.

**Bile pigments; bile salts** – Bile pigments (biliverdin, bilirubin) are the breakdown products of

heme; bile salts are the sodium and potassium salts of bile acids (glycocholate, taurocholate) produced by cholesterol.

**Biliverdin; bilirubin** – Both are bile pigments. Biliverdin is produced from heme in the reticuloendothelial cells; bilirubin is formed by reduction of biliverdin.

**Biotin; biocytin** – Biotin is a B-complex vitamin; biocytin refers to the covalently bound biotin to enzymes (through  $\epsilon$ -amino group of lysine).

**B-Lymphocytes; T-lymphocytes** – B-lymphocytes produce immunoglobulins (antibodies) and are involved in humoral immunity; T-lymphocytes are responsible for cellular immunity.

**Bisphosphate; diphosphate** – Bisphosphate has two phosphates held separately e.g. 2,3-BPG; diphosphate has two phosphates linked together e.g. ADP.

**Calcitriol; calcitonin** – Calcitriol (1,25-DHCC) is the physiologically active form of vitamin D; calcitonin is a peptide hormone, synthesized by thyroid gland.

**Calorimetry; colorimetry** – Calorimetry deals with the measurement of heat production by organism; colorimetry is concerned with the measurement of colour compounds.

**Carboxyl; carbonyl** – These two are functional groups found in organic substances; carboxyl group  $-\text{COOH}$ ; carbonyl  $-\overset{\text{O}}{\text{C}}-$ .

**Carnitine; creatine; creatinine** – Carnitine transports activated fatty acids (acyl CoA) from



- cytosol to mitochondria; creatine is mostly found in the muscle as creatine phosphate, a high energy compound; creatinine is the anhydride of creatine.
- Choline; cholic acid** – Choline is a trimethyl quaternary base and is a constituent of acetylcholine; cholic acid is an important bile acid.
- Chyle; chyme** – Chyle refers to lymph with milky appearance due to chylomicrons; chyme is the partially digested food in the stomach that passes to duodenum.
- Configuration; conformation** – Configuration is the geometric relationship between a given set of atoms (e.g. L- and D-amino acids). Conformation is the special relationship of every atom in a molecule (e.g. secondary structure of protein).
- Cysteine; cystine** – Both are sulfur containing non-essential amino acids. Cysteine contains sulfhydryl (–SH) group; cystine is formed by condensation of two cysteine residues and contains a disulfide (–S–S–) group.
- Dextrins; dextrans; dextrose** – The first two are polysaccharides composed of glucose. Dextrins are the breakdown products of starch; dextrans are gels produced by bacteria from glucose. Dextrose is glucose in solution (dextrorotatory) used in medical practice.
- Diabetes mellitus; diabetes insipidus** – Diabetes mellitus is primarily an impairment in glucose metabolism due to the deficiency of, or inefficient insulin; diabetes insipidus is characterized by excretion of large volumes of urine (polyuria), caused by the deficiency of antidiuretic hormone (ADH).
- Endocytosis; exocytosis** – Endocytosis is the intake of macromolecules by the cells; exocytosis refers to the release of macromolecules from the cells to the outside.
- Epinephrine; norepinephrine** – Both are catecholamines synthesized from tyrosine. Epinephrine is methylated while norepinephrine does not contain a methyl group.
- Exons; introns** – Exons are the DNA sequences coding for proteins; introns are the intervening DNA sequences that do not code for proteins.
- GABA; PABA** –  $\gamma$ -Aminobutyric acid (GABA) is a neurotransmitter; p-aminobenzoic acid (PABA) is a vitamin.
- Gene; genome** – A gene refers to the DNA fragment of a chromosome that codes for a single polypeptide; all the genes of a cell or an organism are collectively known as genome.
- Glu; Gla** – Glu is the code for glutamic acid; Gla is the code for  $\gamma$ -carboxy glutamic acid.
- Glucuronic acid; gluconic acid** – Both are derived from glucose; oxidation of C<sub>6</sub> results in glucuronic acid while oxidation of C<sub>1</sub> yields gluconic acid. Glucuronic acid is produced in uronic acid pathway; gluconic acid is formed in hexose monophosphate shunt.
- Glutaric acid; glutamic acid** – Glutaric acid is a dicarboxylic acid; glutamic acid ( $\alpha$ -amino glutaric acid) is an amino acid.
- Glycogen; glycogenin** – Glycogen is a storage form of carbohydrate (polysaccharide) in the animal body; glycogenin is a protein which serves as a primer for the initiation of glycogen synthesis.
- Glycoproteins; mucoproteins** – Both are conjugated proteins containing carbohydrate as the prosthetic group. The term glycoprotein is used if the carbohydrate content is <4%; mucoprotein contains >4% carbohydrate.
- Hydrophilic; hydrophobic** – Hydrophilic refers to affinity to water; hydrophobic means hatred towards water.
- Insulin; inulin** – Insulin is a peptide hormone; inulin is a polysaccharide composed of fructose.
- In vivo; in vitro** – In vivo refers to within the cell or organism; in vitro means in the test tube.
- Isoniazid; iproniazid** – Isoniazid is an anti-tuberculosis drug; iproniazid is an anti-depressant drug.
- Lactam; lactim** – These terms are used to represent tautomerism. Lactam indicates the existence of a molecule in keto form; lactim represents a molecule in enol form.
- Lactose; lactase** – Lactose is a disaccharide; lactase is an enzyme that cleaves lactose to glucose and galactose.
- Linoleic acid; linolenic acid** – Both are 18 carbon unsaturated fatty acids. Linoleic acid has two double bonds; linolenic acid has three double bonds.
- Lipoproteins; lipotropic factors** – Lipoproteins are molecular complexes composed of lipids and proteins; lipotropic factors are the substances (e.g. choline, betaine), the deficiency of which causes accumulation of fat in liver.

**$\beta$ -Lipoprotein;  $\beta$ -lipotropin** –  $\beta$ -Lipoprotein refers to the low density lipoproteins;  $\beta$ -lipotropin is a peptide hormone derived from pro-opiomelanocortin (POMC) peptide.

**Lyases; ligases** – Lyases are the enzymes that catalyse the addition or removal of water, ammonia,  $\text{CO}_2$  etc.; ligases catalyse the synthetic reactions where two molecules are joined together.

**Malate; malonate; mevalonate** – Malate is an intermediate in the citric acid cycle; malonate is a competitive inhibitor of the enzyme succinate dehydrogenase; mevalonate is an intermediate in cholesterol biosynthesis.

**Melanin; melatonin** – Melanin is the pigment of skin and hair; melatonin is a hormone synthesized by pineal gland.

**Maltose; maltase** – Maltose is a disaccharide; maltase is an enzyme that cleaves maltose to two molecules of glucose.

**Methyl, methenyl; methylene** – All the three are one-carbon fragments as shown in brackets, methyl ( $-\text{CH}_3$ ); methenyl ( $-\text{CH}=\text{}$ ); methylene ( $-\text{CH}_2-$ ).

**Molarity; molality** – Molarity is defined as the number of moles of a solute per liter solution; molality represents the number of moles of a solute per 1,000 g of solvent.

**Nicotinic acid; nicotine** – Nicotinic acid is a B-complex vitamin; nicotine is an alkaloid present in tobacco leaves.

**Nucleoside; nucleotide** – A nucleoside is composed of a nitrogen base and a sugar; nucleotide contains one or more phosphate groups bound to nucleoside.

**Osmolarity; osmolality** – Osmolarity represents osmotic pressure exerted by the number of moles (milli moles) per liter solution; osmolality refers to the osmotic pressure exerted by the number of moles (milli moles) per kg solvent.

**Palmitate; palmitoleate** – Both are even chain (16-carbon) fatty acids. Palmitate is a saturated fatty acid; palmitoleate is a monounsaturated fatty acid.

**Phosphatidyl ethanolamine; phosphatidyl ethanolamine** – Both are phospholipids. In phosphatidyl ethanolamine, the fatty acid is bound by an ester linkage. The fatty acid is

held by an ether linkage in phosphatidyl ethanolamine.

**Phytic acid; phytanic acid** – Phytic acid is formed by the addition of six phosphate molecules to inositol, it is an inhibitor of the intestinal absorption of calcium and iron; phytanic acid is an unusual fatty acid derived from phytol, a constituent of chlorophyll.

**Prokaryotes; eukaryotes** – Prokaryotes are the cells that lack a well defined nucleus; eukaryotes possess a well-defined nucleus.

**Prolamines; protamines** – Both are simple proteins. Prolamines are soluble in alcohol; protamines are basic protein soluble in  $\text{NH}_4\text{OH}$ .

**Pyridine; pyrimidine; pteridine** – All the three are heterocyclic rings containing nitrogen, as depicted below.



Pyridine ring is found in niacin and pyridoxine; pyrimidine is present in thiamine (vitamin  $\text{B}_1$ ), thymine, cytosine and uracil; folic acid contains pteridine ring.

**Pyridoxine; pyridoxal** – Pyridoxine is the primary alcohol form of vitamin  $\text{B}_6$ ; pyridoxal is the aldehyde form of  $\text{B}_6$ .

**RDA; SDA** – RDA (recommended dietary/daily allowance) represents the quantities of nutrients to be provided in the diet daily for maintenance of good health and physical efficiency; specific dynamic action (SDA) is the extra heat produced by the body over and above the caloric value of foodstuffs.

**Renin; Rennin** – Renin is synthesized by the kidneys and is involved in vasoconstriction causing hypertension; rennin is an enzyme found in gastric juice responsible for coagulation of milk.

**Ribosomes; ribozymes** – Ribosomes are the sites of protein biosynthesis; ribozymes refer to the RNA molecules which function as enzymes.

**Retinol; retinal** – Retinol is the alcohol form of vitamin A; retinal is the aldehyde form obtained by the oxidation of retinol.



- Scleroproteins; selenoproteins** – Scleroproteins are a group of fibrous proteins; selenoproteins contain the amino acid selenocysteine.
- Serotonin; melatonin** – Serotonin is a neurotransmitter synthesized from tryptophan; melatonin is a hormone derived from serotonin in the pineal gland.
- Somatotropin; somatostatin; somatomedin** – Somatotropin is the other name for growth hormone (GH); growth hormone release inhibiting hormone (GRIH) is also called somatostatin; somatomedin refers to the insulin-like growth factor -I (IGF-I), produced by liver in response to GH action.
- Sucrose; sucrase** – Sucrose is a disaccharide; sucrase is an enzyme that cleaves sucrose to glucose and fructose.
- Synthase; synthetase** – Both the enzymes are concerned with biosynthetic reactions. Synthase does not require ATP; synthetase is dependent on ATP for energy supply. (**Note** : This distinction between synthase and synthetase however, is not maintained strictly by most authors).
- Thiamine; thymine** – Thiamine is a vitamin ( $B_1$ ); thymine is a pyrimidine base found in DNA structure.
- Thiokinase; thiolase** – Thiokinase activates fatty acids to acyl CoA; Thiolase catalyses the final reaction in  $\beta$ -oxidation to liberate acetyl CoA from acyl CoA.
- Transcription; translation** – Transcription refers to the synthesis of RNA from DNA; translation involves the protein synthesis from the RNA.
- Uric acid; uronic acid** – Uric acid is the end product of purine metabolism; uronic acids are formed by the oxidation of aldehyde group of monosaccharides (e.g. glucuronic acid).
- Ureotelic; uricotelic** – Ureotelic organisms (e.g. mammals) convert  $NH_3$  to urea; uricotelic organisms (e.g. reptiles) convert  $NH_3$  to uric acid.
- Vitamin A; coenzyme A** – Vitamin A is fat soluble vitamin; coenzyme A is derived from water soluble vitamin, pantothenic acid.



# Appendix V : Practical Biochemistry—Principles

## QUALITATIVE EXPERIMENTS

Several laboratory qualitative experiments are performed to identify the compounds of biochemical importance (carbohydrates, proteins/amino acids, non-protein nitrogenous substances) and to detect the abnormal constituents of urine. The principles of the reactions pertaining to the most widely employed qualitative tests are described here.

### I. REACTIONS OF CARBOHYDRATES

The carbohydrates used in the laboratory for the qualitative tests include glucose and fructose (monosaccharides), sucrose, lactose and maltose (disaccharides) and starch (polysaccharide). The principles of the reactions of carbohydrates are given :

1. **Molisch test** : It is a general test for the detection of **carbohydrates**. The strong  $H_2SO_4$  hydrolyses carbohydrates (poly- and disaccharides) to liberate monosaccharides. The monosaccharides get dehydrated to form furfural (from pentoses) or hydroxy methylfurfural (from hexoses) which condense with  $\alpha$ -naphthol to form a violet coloured complex.

2. **Iodine test** : Polysaccharides combine with iodine to form a coloured complex. Thus, **starch** gives blue colour while dextrins give red colour with iodine.

3. **Benedict's test** : This is a test for the identification of **reducing sugars**, which form enediols (predominantly under alkaline conditions). The enediol forms of sugars reduce cupric ions ( $Cu^{2+}$ ) of copper sulfate to cuprous ions ( $Cu^+$ ) which form a yellow precipitate of cuprous hydroxide or a red precipitate of cuprous oxide.

4. **Barfoed's test** : The principle of this test is the same as that of Benedict's test except that the

reduction is carried out in mild acidic medium. Since acidic medium is not favourable for reduction, only strong reducing sugars (monosaccharides) give this test positive. Thus, Barfoed's test serves as a key reaction to distinguish **monosaccharides** from disaccharides.

5. **Seliwanoff's test** : This is a specific test for **ketohexoses**. Concentrated hydrochloric acid dehydrates ketohexoses to form furfural derivatives which condense with resorcinol to give a cherry red complex.

6. **Foulger's test** : This is also a test for **ketohexoses**. The furfural derivatives formed from ketohexoses condense with urea in the presence of stannous chloride to give a blue colour.

7. **Rapid furfural test** : **Ketohexoses** are converted to furfural derivatives by HCl which form a purple colour complex with  $\alpha$ -naphthol.

8. **Osazone test** : Phenylhydrazine in acetic acid, when boiled with reducing sugars forms osazones. The first two carbons ( $C_1$  and  $C_2$ ) are involved in this reaction. The sugars that differ in their configuration on these two carbons give the same type of osazones, since the difference is marked by binding with phenylhydrazine. Thus, glucose, fructose and mannose give the same type (needle shaped) of osazones. However, the osazones of reducing disaccharides differ — maltose gives sunflower-shaped while lactose powder-puff shaped.

9. **Sucrose hydrolysis test** : Sucrose is a non-reducing sugar, hence it does not give Benedict's and Barfoed's tests. Sucrose can be hydrolysed by concentrated HCl, to be converted to glucose and fructose (reducing monosaccharides) which answer the reducing reactions. However, after sucrose hydrolysis, the medium has to be made alkaline (by adding  $Na_2CO_3$ ) for effective reduction process.

## II. REACTIONS OF PROTEINS

The proteins employed in the laboratory for the qualitative tests include albumin, globulins, casein, gelatin and peptones. The principle of the most common reactions of proteins/amino acids performed in the laboratory are given hereunder.

### A. PRECIPITATION REACTIONS

Proteins exist in colloidal solution due to hydration of polar groups ( $-\text{COO}^-$ ,  $-\text{NH}_3^+$ ,  $-\text{OH}$ ). They can be precipitated by dehydration or neutralization of polar groups. Several methods are in use to achieve protein precipitation.

**1. Precipitation by neutral salts :** The process of protein precipitation by the addition of neutral salts such as ammonium sulfate or sodium sulfate is referred to as salting out. This phenomenon is explained on the basis of dehydration of protein molecules by salts. This causes increased protein-protein interaction, resulting in molecular aggregation and precipitation.

The amount of salt required for protein precipitation depends on the size (molecular weight) of the protein molecule. In general, the higher is the protein molecular weight, the lower is the salt required for precipitation. Thus, serum globulins are precipitated by half saturation with ammonium sulfate while albumin is precipitated by full saturation.

**2. Precipitation by salts of heavy metals :** Heavy metal ions like  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  cause precipitation of proteins. These metals being positively charged, when added to protein solution (negatively charged) in alkaline medium result in precipitate formation.

**3. Precipitation by anionic or alkaloid reagents :** Proteins can be precipitated by trichloroacetic acid, sulphosalicylic acid, phosphotungstic acid, picric acid, tannic acid, phosphomolybdic acid etc. By the addition of these acids, the proteins existing as cations are precipitated by the anionic form of acids to produce protein-sulphosalicylate, protein-tungstate, protein-picrate etc.

The anionic reagents such as phosphotungstic acid and trichloroacetic acid are used to prepare protein-free filtrate of blood needed for several estimations (e.g., urea, sugar) in the laboratory.

**4. Precipitation by organic solvents :** Organic solvents such as alcohol are good protein

precipitating agents. They dehydrate the protein molecule by removing that water envelope and cause precipitation.

### B. COLOUR REACTIONS

The proteins give several colour reactions which are often useful to identify the nature of the amino acids present in them as shown in the table.

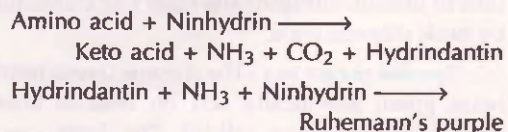
#### Colour reactions of proteins/amino acids

| Reaction                     | Specific group or amino acid                         |
|------------------------------|--|
| 1. Biuret reaction           | Two peptide linkages                                 |
| 2. Ninhydrin reaction        | $\alpha$ -Amino acids                                |
| 3. Xanthoproteic reaction    | Benzene ring of aromatic amino acids (Phe, Tyr, Trp) |
| 4. Millons reaction          | Phenolic group (Tyr)                                 |
| 5. Hopkins-Cole reaction     | Indole ring (Trp)                                    |
| 6. Sakaguchi reaction        | Guanidino group (Arg)                                |
| 7. Nitroprusside reaction    | Sulfhydryl groups (Cys)                              |
| 8. Sulfur test               | Sulfhydryl groups (Cys)                              |
| 9. Pauly's test              | Imidazole ring (His)                                 |
| 10. Folin-Coicallteau's test | Phenolic groups (Tyr)                                |

**1. Biuret reactions :** Biuret is a compound formed by heating urea to  $180^\circ\text{C}$ . When biuret is treated with dilute copper sulfate in alkaline medium, a purple colour is obtained. This is the basis of biuret test used for identification of proteins and peptides.

Biuret test is answered by compounds containing two or more  $\text{CO}-\text{NH}$  groups i.e., **peptide bonds**. All proteins and peptides possessing atleast two peptide linkages i.e., tripeptides (with 3 amino acids) give positive biuret test. The principle of biuret test is conveniently used to detect the presence of proteins in biological fluids. The mechanism of biuret test is not clearly known. It is believed that the colour is due to the formation of a copper co-ordinated complex.

**2. Ninhydrin reaction :** The  $\alpha$ -amino acids react with ninhydrin to form a purple, blue or pink colour complex (Ruhemann's purple).





3. **Xanthoproteic reaction** : Xanthoproteic reaction is due to nitration of **aromatic amino acids** (tryptophan, tyrosine and phenylalanine) on treatment with strong nitric acid at high temperature.

4. **Millon's test** : This test is given by the amino acid **tyrosine**, or any other compound containing hydroxyphenyl ring. A red colour or precipitate is obtained in this reaction due to the formation of mercury complex of nitrophenol derivative.

5. **Hopkins-Cole reaction** : This reaction is specific for the indole ring of **tryptophan**. It combines with formaldehyde in the presence of the oxidizing agent (sulfuric acid with mercuric sulfate) to form a violet or purple coloured compound.

6. **Sakaguchi reaction** : Arginine, containing guanidino group, reacts with  $\alpha$ -naphthol and alkaline hypobromite to form a red colour complex.

7. **Sulfur test** : This is a test specific for sulfur containing amino acids namely **cysteine** and **cystine**, but not methionine. When cysteine and cystine are boiled with sodium hydroxide, organic sulfur is converted to inorganic sodium sulfide. This reacts with lead acetate to form a black precipitate of lead sulfide. Methionine does not give this test, since sulfur of methionine is not split by alkali.

8. **Pauly's test** : This reaction is specific for **histidine** (imidazole ring). Diazotised sulfanilic acid reacts with imidazole ring in alkaline medium to form a red coloured complex.

9. **Molisch test** : This is a specific test for the detection of **carbohydrates**. The proteins containing carbohydrates (e.g., glycoproteins) give this test positive. Albumin contains carbohydrate bound to it, hence answers Molisch test.

### III. REACTIONS OF NON-PROTEIN NITROGENOUS SUBSTANCES

The non-protein nitrogenous (NPN) substances of biochemical importance include urea, uric acid and creatinine.

1. **Sodium hypobromite test** : This is a test for the detection of **urea**. Sodium hypobromite decomposes urea to liberate nitrogen. The latter can be identified by brisk effervescence.

2. **Specific urease test** : The enzyme urease (source-horse gram) specifically acts on **urea** to liberate ammonium carbonate (alkali). The latter can be

identified by a colour change in phenolphthalein indicator (pink colour in alkaline medium).

3. **Benedict's uric acid test** : **Uric acid**, being a strong reducing agent, reduces phosphotungstate to tungsten blue in alkaline medium.

4. **Murexide test** : **Uric acid** is oxidized by nitric acid to give purpuric acid (reddish yellow). This in turn combines with ammonia to form purple red colour ammonium purpurate (murexide).

5. **Jaffe's test** : Creatinine reacts with picric acid in alkaline medium to form orange red colour complex.

### IV. ABNORMAL CONSTITUENTS OF URINE

Urine is the most important excretory fluid from the body. Some of the diseases are associated with an excretion of abnormal constituents in urine. The identification of such compounds in urine is of great diagnostic importance.

| Urine abnormal constituent | Associated disorder(s)                     |
|----------------------------|--|
| Albumin                    | Kidney damage (glomerulonephritis)         |
| Hemoglobin                 | Damage to kidneys or urinary tract.        |
| Glucose                    | Diabetes mellitus, renal glycosuria.       |
| Ketone bodies              | Diabetes mellitus, starvation.             |
| Bile salts                 | Obstructive jaundice                       |
| Bile pigments              | Obstructive jaundice and hepatic jaundice. |

1. **Sulfosalicylic acid test** : **Proteins** get precipitated by sulfosalicylic acid by forming protein-sulfosalicylate.

2. **Heat coagulation test** : This is a test for the detection of **albumin** and/or **globulins** in urine. Heat coagulation test is based on the principle of denaturation of proteins, followed by coagulation.

(Note : Small amounts of dilute acetic acid are added to dissolve the phosphates and sulfates that get precipitated on heating.)

3. **Benzidine test** : This test detects the presence of **blood**. Hemoglobin (acts like peroxidase) decomposes hydrogen peroxide to liberate nascent oxygen (O<sup>-</sup>) which oxidises benzidine to a green or blue coloured complex.

(Note : Pus cells of urine possess peroxidase activity which interferes in benzidine test. This can be eliminated by boiling the urine prior to the test to inactivate the enzyme).



4. **Benedict's test** : This is a semiquantitative test for the detection of urine reducing sugars (primarily glucose). Benedict's test is based on the principle of reducing property of sugars (described in detail under reactions of carbohydrates). Colour of the precipitate formed indicates the approximate amount of **glucose** present in urine. Thus, green turbidity = traces; green precipitate = 0.5%; yellow precipitate = 1%; orange precipitate = 1.5% brick red precipitate = 2%. (Note : Benedict's test is not specific to glucose, since it can be answered by any reducing substance).

5. **Glucose oxidase test** : This is a strip test for the **specific detection of glucose**. The enzyme glucose oxidase oxidizes glucose to liberate hydrogen peroxide which in turn is converted to nascent oxygen (O-) by peroxidase enzyme. The compound O-dianisidine combines with nascent oxygen to form a coloured (yellow to red) complex.

6. **Rothera's test** : Nitroprusside in alkaline medium reacts with keto group of **ketone bodies** (acetone and acetoacetate) to form a purple ring. This test is not given by  $\beta$ -hydroxybutyrate.

7. **Hay's test** : This test is based on the surface tension lowering property of **bile salts** (sodium glycocholate and sodium taurocholate). Sulfur powder sprinkled on the surface of urine containing bile salts sinks to the bottom.

8. **Petternkofer's test** : This test is employed for the detection of **bile salts**. The furfural derivatives (by reacting sugar with concentrated  $H_2SO_4$ ) condense with bile salts to form a purple ring.

9. **Gmelin's test** : Nitric acid oxidizes the bile pigment bilirubin to biliverdin (green) or bilicyanin (blue). Gmelin's test gives a play of colours and is used for the identification of **bile pigments**.

10. **Fouchet's test** : This test is also employed for the detection of **bile pigments**. Bile pigments are adsorbed on barium sulfate. Fouchet's reagents (containing ferric chloride in trichloroacetic acid) oxidizes bilirubin to biliverdin (green) and bilicyanin (blue).

## QUANTITATIVE EXPERIMENTS

Quantitative experiments, dealing with the determination of concentrations of several biologically important compounds and the assay of many enzymes, are of great significance in the laboratory practice. Very often, the ultimate diagnosis and prognosis of a large number of diseases are guided by the quantitative biochemical investigations.

The principles involved in some of the quantitative experiments, commonly employed in the biochemistry laboratory by an undergraduate student, are briefly described here.

### 1. Blood glucose estimation

The quantitative determination of blood (plasma/serum) glucose is of great importance in the diagnosis and monitoring of diabetes mellitus.

- (i) **Folin Wu method** : Alkaline copper (cupric ions) is reduced by glucose when boiled with protein free blood filtrate to cuprous oxide. The cuprous oxide in turn reacts with phosphomolybdic acid to form blue coloured oxides of molybdenum. The intensity of the colour can be measured in a colorimeter at a wavelength 680 nm. [Folin Wu method is rather old and is not specific for glucose determination, since other substances (e.g., fructose, lactose, glutathione) also bring about reduction. Consequently the blood glucose level when estimated by Folin Wu method is higher i.e., normal fasting is 80-120 mg/dl against true glucose 60-100 mg/dl]
- (ii) **O-Toluidine method** : Glucose combines with O-toluidine when boiled in acid medium to form a green coloured complex which can be measured in a colorimeter at a wavelength 630 nm. (This method determines glucose alone).
- (iii) **Glucose oxidase-peroxidase (GOD—POD) method** : This is an enzymatic determination of blood glucose. Glucose gets oxidized by glucose oxidase to

gluconic acid and hydrogen peroxide. The enzyme peroxidase converts hydrogen peroxide to water and oxygen. The oxygen in turn reacts with 4-aminophenone in the presence of phenol to form a pink coloured complex, the intensity of which can be measured at 530 nm.

## 2. Blood urea estimation

Determination of blood urea (reference range 10-40 mg/dl) is important for the evaluation of kidney (renal) function. Elevation of blood urea is associated with pre-renal (diabetic coma, thyrotoxicosis), renal (acute glomerulonephritis, polycystic kidney) and post-renal (obstruction in the urinary tract, due to tumors, stones) conditions.

**Diacetyl monoxime (DAM) method :** Urea when heated with diacetyl monoxime forms a yellow coloured complex of dioxime derivatives which can be measured at 520 nm.

## 3. Serum creatinine estimation

Estimation of serum creatinine (reference range 0.5-1.5 mg/dl) is used as a diagnostic test to assess kidney function. Serum creatinine is not influenced by endogenous and exogenous factors, as is the case with urea. Hence, some workers consider serum creatinine as a more reliable indicator of renal function.

**Alkaline picrate method :** This method is based on Jaffe's reaction. Creatinine reacts with alkaline picrate to form creatinine picrate, an orange red coloured complex, which can be measured in a colorimeter at 530 nm.

(Note : Urinary creatinine can also be determined by employing the same principle given above).

## 4. Determination of serum proteins

The normal concentration of total serum proteins is in the range 6-8 g/dl (albumin 3.5-5.0 g/dl; globulins 2.5-3.5 g/dl; A/G ratio is 1.2 to 1.5 : 1). The A/G ratio is lowered either due to a decrease in albumin or an increase in globulins.

Serum albumin concentration is decreased in liver diseases, severe protein malnutrition, and excretion of albumin in urine (due to renal damage). Serum globulin concentration is elevated in chronic infections and multiple myeloma.

**Biuret method :** Peptide bonds ( $-\text{CO}-\text{NH}$ ) of proteins react with cupric ions in alkaline medium to form a violet colour complex which is measured at a wavelength 530 nm. This method is suitable for total serum proteins with estimation.

**Bromocresol green (BCG) dye method :** This technique is employed for the estimation of serum albumin. BCG dye reacts with albumin to form an intense blue-green coloured complex which can be measured at 628 nm.

## 5. Estimation of serum bilirubin

The total bilirubin concentration in serum is 0.2-1 mg/dl (conjugated ~ 0.6 mg/dl; unconjugated ~ 0.4 mg/dl). Elevation in serum bilirubin concentration is observed in jaundice. Unconjugated bilirubin is increased in hemolytic jaundice, conjugated bilirubin in obstructive jaundice, while both of them are increased in hepatic jaundice.

**van den Bergh reaction :** Serum bilirubin estimation is based on van den Bergh reaction. The principle of the reaction is that diazotised sulfanilic acid (formed by mixing equal volumes of sulfanilic acid in HCl and sodium nitrite) reacts with bilirubin to form a purple coloured azobilirubin which can be measured at 540 nm.

## 6. Estimation of serum cholesterol

Serum cholesterol concentration (reference range 150-225 mg/dl) is elevated in atherosclerosis, diabetes mellitus, obstructive jaundice and hypothyroidism. Decreased levels are observed in hyperthyroidism.

**Acetic anhydride method :** Serum cholesterol reacts with acetic anhydride in the presence of glacial acetic acid and concentrated  $\text{H}_2\text{SO}_4$  to form a green coloured complex. Intensity of this colour is measured at 560 nm.

## 7. Estimation of serum uric acid

Uric acid is the end product of purine metabolism. Its concentration in serum is increased (reference range - men 4-8 mg/dl; women 3-6 mg/dl) in gout.

**Henry-Caraway's method :** Uric acid in the protein-free filtrate when treated with phosphotungstic acid in the presence of sodium carbonate (alkaline solution) gives a blue coloured complex which can be measured at 660 nm.



### 8. Estimation of serum calcium

Serum calcium level is elevated (reference range 9-11 mg/dl) in hyperparathyroidism and decreased in hypothyroidism.

#### ***O*-Cresolphthalein complexone method :**

Calcium reacts with the dye, *O*-cresolphthalein complexone (CPC) in alkaline solution to form a complex which can be measured at a wavelength 660 nm.

### 9. Estimation of serum phosphorus (inorganic)

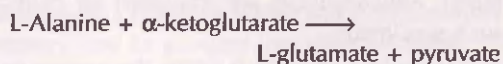
Serum phosphate (reference range 3-4.5 mg/dl) is increased in hypoparathyroidism, and decreased in hyperparathyroidism and renal rickets.

For the determination of serum phosphate, serum proteins are precipitated by trichloroacetic acid. The protein-free filtrate containing inorganic phosphate is reacted with molybdic acid reagent to form phosphomolybdate. The latter in turn is reduced to molybdenum blue by treatment with 1-amino 2-naphthol-4 sulfonic acid (ANSA). The intensity of the blue colour is measured at 689 nm.

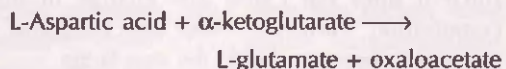
### 10. Determination of SGPT and SGOT

Serum glutamate pyruvate transaminase (SGPT; alanine transaminase) and serum glutamate oxaloacetate transaminase (SGOT; aspartate transaminase) are two important diagnostic enzymes. SGPT activity (reference range 5-40 IU/L) is more specifically increased in liver diseases (hepatic jaundice). SGOT activity is elevated (reference range 5-45 IU/L) in heart diseases (myocardial infarction).

**Principle of assay :** SGPT catalyses the following reaction



SGOT brings about the following reaction



The keto acid (pyruvate or oxaloacetate), formed in the above reaction, when treated with 2, 4-dinitrophenyl hydrazine forms dinitrophenyl hydrozone (brown colour) in alkaline medium which can be measured at 505 nm.

### 11. Determination of serum alkaline phosphatase

The activity of the enzyme serum alkaline phosphatase (normal range 3-13 KA Units/dl) is elevated in rickets and obstructive jaundice.

**Principle of assay :** Alkaline phosphatase hydrolyses disodium phenylphosphate liberating phenol. On treatment with 4-amino antipyrine in alkaline medium, phenol gives ferricyanide (reddish colour) which can be measured at 520 nm.

### 12. Determination of serum amylase

Serum amylase activity is increased (reference range 80-180 Somogyi Units/dl) in acute pancreatitis.

**Principle of assay :** Amylase acts on starch and hydrolyses to dextrans and maltose. Starch forms blue coloured complex with iodine, a decrease in the colour (measured at 670 nm) is proportional to the activity of amylase.

### 13. Analysis of cerebrospinal fluid

Cerebrospinal fluid (CSF) is the aqueous medium surrounding the brain and spinal cord. From the biochemical perspective, estimation of proteins and glucose in CSF is important. Increase in protein (reference range 15-40 mg/dl) and decrease in glucose (reference range 50-75 mg/dl) in the cerebrospinal fluid are observed in tuberculosis meningitis.

**CSF protein estimation :** Sulfosalicylic acid (in sodium sulfate solution) precipitates CSF proteins and the turbidity is measured at 680 nm.

**CSF glucose estimation :** Any one of the standard methods employed for the determination of blood glucose (already described) can be used for CSF glucose estimation.



## Appendix VI : Clinical Biochemistry Laboratory

The ultimate application of the biochemistry subject is for the health and welfare of mankind. Clinical biochemistry (also known as **clinical chemistry** or **chemical pathology**) is the laboratory service absolutely essential for medical practice. The results of the biochemical investigations carried out in a clinical chemistry laboratory will help the clinicians to determine the diseases (diagnosis) and for follow-up of the treatment/recovery from the illness (prognosis). Biochemical investigations hold the key for the diagnosis and prognosis of diabetes mellitus, jaundice, myocardial infarction, gout, pancreatitis, rickets, cancers, acid-base imbalance etc. Successful medical practice is unimaginable without the service of clinical biochemistry laboratory.

The **biological fluids** employed in the clinical biochemistry laboratory include **blood, urine, cerebrospinal fluid** and pleural fluid. Among these, blood (directly or in the form of plasma or serum) is frequently used for the investigations in the clinical biochemistry laboratory.

### COLLECTION OF BLOOD

Venous **blood** is most commonly used for a majority of biochemical investigations. It can be drawn from any prominent vein (usually from a vein on the front of the elbow). **Capillary blood** (<0.2 ml) obtained from a finger or thumb, is less frequently employed. **Arterial blood** (usually drawn under local anesthesia) is used for blood gas determinations.

**Precautions for blood collection :** Use of sterile (preferably disposable) needles and syringes, cleaning of patients skin, blood collection in clean and dry vials/tubes are some of the important precautions.

### CHOICE OF BLOOD SPECIMENS

Biochemical investigations can be performed on 4 types of blood specimens—whole blood, plasma, serum and red blood cells. The selection of the specimen depends on the parameter to be estimated. **Whole blood** (usually mixed with an anticoagulant) is used for the estimation of hemoglobin, carboxyhemoglobin, pH, glucose, urea, non-protein nitrogen, pyruvate, lactate, ammonia etc. (Note : for glucose determination, plasma is preferred in recent years).

**Plasma**, obtained by centrifuging the whole blood collected with an anticoagulant, is employed for the parameters—fibrinogen, glucose, bicarbonate, chloride, ascorbic acid etc.

**Serum** is the supernatant fluid that can be collected after centrifuging the clotted blood. It is the most frequently used specimen in the clinical biochemistry laboratory. The parameters estimated in serum include proteins (albumin/globulins), creatinine, bilirubin, cholesterol, uric acid, electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ), enzymes (ALT, AST, LDH, CK, ALP, ACP, amylase, lipase) and vitamins.

**Red blood cells** are employed for the determination of abnormal hemoglobins, glucose 6-phosphate dehydrogenase, pyruvate kinase etc.

### ANTICOAGULANTS

Certain biochemical tests require unclotted blood. Anticoagulants are employed for collecting such specimens.

**Heparin :** Heparin (inhibits the conversion of prothrombin to thrombin) is an ideal anticoagulant, since it does not cause any change in blood composition. However, other anticoagulants are preferred to heparin, due to the cost factor.

**Potassium or sodium oxalate :** These compounds precipitate calcium and inhibit blood coagulation. Being more soluble, potassium oxalate (5-10 mg per 5 ml blood) is preferred.

**Potassium oxalate and sodium fluoride** : These anticoagulants are employed for collecting blood to estimate glucose. Further sodium fluoride inhibits glycolysis and preserves blood glucose concentration.

**Ammonium oxalate and potassium oxalate** : A mixture of these two compounds in the ratio 3 : 2 is used for blood collection to carry out certain hematological tests.

**Ethylene diaminetetracetic acid (EDTA)** : It chelates with calcium and blocks coagulation. EDTA is employed to collect blood for hematological examinations.

## HEMOLYSIS

The rupture or lysis of RBC, releasing the cellular constituents interferes with the laboratory investigations. Therefore, utmost care should be taken to avoid hemolysis when plasma or serum are used for biochemical tests. Use of dry syringes, needles and containers, allowing slow flow of blood into syringe are among the important precautions to avoid hemolysis.

## PRESERVATION OF BLOOD SPECIMENS

Plasma or serum should be separated within 2 hours after blood collection. It is ideal and advisable to analyse blood, plasma or serum, immediately after the specimen collection. This however, may not be always possible. In such a case, the samples (usually plasma/serum) can be stored at 4°C until analysed. For enzyme analysis, the sample are preserved at -20°C.

## TYPES OF LABORATORY TESTS

The biochemical investigations (on blood/plasma/serum) carried out in the clinical biochemistry laboratory may be grouped into different types.

**1. Discretionary or on-off tests** : Most common clinical biochemistry tests that are designed to answer specific questions. e.g., does the patient have increased blood urea/glucose concentration? Normally, these tests are useful to support the diagnosis.

**2. Biochemical profiles** : These tests are based on the fact that more useful information on the patients disease status can be obtained by analysing more constituents rather than one e.g., **plasma electrolytes** (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, bicarbonate, urea); **liver function tests** (serum bilirubin, ALT, AST).

**3. Dynamic function tests** : These tests are designed to measure the body's response to external stimulus e.g., oral glucose tolerance test (to assess glucose homeostasis) ; bromosulphthalein test (to assess liver function).

**4. Screening tests** : These tests are commonly employed to identify the inborn errors of metabolism, and to check the entry of toxic agents (pesticides, lead, mercury) into the body.

**5. Metabolic work-up tests** : The programmed intensive investigations carried out to identify the endocrinological disorders come under this category.

The term **emergency tests** is frequently used in the clinical laboratory. It refers to the tests to be performed immediately to help the clinician for proper treatment of the patient e.g., blood glucose, urea, serum electrolytes.

## COLLECTION OF URINE

Urine, containing the metabolic waste products of the body in water is the most important excretory fluid. For biochemical investigations, urine can be collected as a single specimen or for 24 hours. Single specimens of urine, normally collected in the morning, are useful for qualitative tests e.g., sugar, proteins. Twenty four hour urine collections (done between 8 AM to 8 AM) are employed for quantitative estimation of certain urinary constituents e.g., proteins, hormones, metabolites.

**Preservatives for urine** : For the collection of 24 hr urine samples, preservatives have to be used or else urine undergoes changes due to bacterial action. Hydrochloric acid, toluene, light petroleum, thymol, formalin etc., are among the common preservatives used.

## CEREBROSPINAL FLUID (CSF)

CSF is a fluid of the nervous system. It is formed by a process of selective dialysis of plasma by the choroid plexuses of the ventricles of the brain. The total volume CSF is 100-200 ml.

**Collection of CSF** : CSF is collected by puncturing the interspace between the 3rd and the 5th lumbar vertebrae, under aseptic conditions and local anesthesia.



**Biochemical investigations on CSF :** Protein, glucose and chloride estimations are usually performed in the clinical biochemistry laboratory.

### QUALITY CONTROL

Quality control in clinical biochemistry laboratory refers to the reliability of investigative service. Any error in the laboratory will jeopardize the lives of patients. It is therefore utmost important that the laboratory errors are identified and rectified.

Quality control comprises of four interrelated factors namely precision, accuracy, specificity and sensitivity.

**Precision** refers to the reproducibility of the result when the same sample is analysed on different occasions (replicate measurements) by the same person. For instance, the precision is good, if the blood glucose level is 78, 80 and 82 mg/dl on replicates.

**Accuracy** means the closeness of the estimated result to the true value e.g., if true blood urea level is 50 mg/dl, the laboratory reporting 45 mg/dl is more accurate than the one reporting 35 mg/dl.

**Specificity** refers to the ability of the analytical method to specifically determine a particular parameter e.g., glucose can be specifically estimated by enzymatic glucose oxidase method.

**Sensitivity** deals with the ability of a particular method to detect small amounts of the measured constituent.

### METHODS OF QUALITY CONTROL

**Internal quality control** refers to the analysis of the same pooled sample on different days in a

laboratory, the results should vary within a narrow range.

**External quality control** deals with the analysis of a sample received from outside, usually from a national or regional quality control centre. The results obtained are then compared.

### AUTOANALYSERS IN CLINICAL CHEMISTRY

The heavy work load in the clinical biochemistry laboratory has lead to the discovery of autoanalysers. These modern equipment are useful to analyse hundreds of samples in a short time. Single channel and multi-channel machines (autoanalysers) based on the principles of either continuous or discrete analysis are available on the market.

### ANALYSIS IN CLINICAL BIOCHEMISTRY LABORATORY AND REFERENCE VALUES

As already stated, clinical biochemistry laboratory is a service-oriented establishment for the benefit of patient health care. The reader may refer tools of biochemistry (**Chapter 41**) and principles of practical biochemistry (**Appendix-V**) for a brief knowledge on the principles of some of the equipment used and the laboratory investigations employed.

The details on the biochemistry of health and disease states in relation to the normal and abnormal biochemical data are described in the text of this book. For ready reference, the most common reference biochemical values are given on the inside of back cover.



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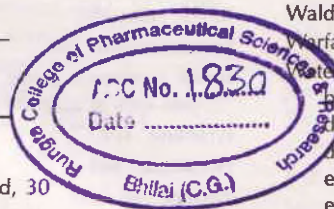
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e-mail : books@cal.vsnl.net.in

ISBN 81-87134-80-1



9 788187 134800