APPENDIX
APPENDIX

A list consisting of the methods for the preparation of various reagents used is given below:

Fixatives

**Zenker's fluid**

Stock solution was prepared by adding:

- Potassium dichromate = 2.5 g
- Mercuric chloride = 5.0 g
- Distilled water = 100 ml

In 20 ml of this solution was added 1 ml of acetic acid just before use.

**Bouin's fluid**

This was prepared by mixing the following:

- Saturated aqueous solution of picric acid = 75 ml
- 40% formaldehyde = 25 ml
- Glacial acetic acid = 5 ml

**Formaldehyde calcium**

The fixative was prepared by adding:

- Calcium chloride = 1 g
- 10% formalin = 10 ml
- H₂O = 100 ml
The solution was made from formalin neutralized with marble chips.

**Weak Bouins**

The fixative was prepared by mixing:

- Formalin = 10 ml
- Saturated picric acid = 50 ml
- Glacial acetic acid = 5 ml
- Distilled water = 35 ml

**STAINS**

**Iron haematoxylin**

This stain was prepared by adding 250 mg of haematoxylin and 25 mg of sodium iodate to 47.5 ml of boiling distilled water. On cooling 25 ml of absolute alcohol was added.

**Schiff's reagent**

200 ml of distilled water was boiled and in it 1 g of basic fuschin was dissolved. The solution was cooled to 50°C and was filtered. To the filtrate was added 20 ml of 1N HCl. After cooling to 25°C, 1 g of sodium metabisulphite was dissolved in it. The mixture was left in the dark for
overnight. After 24 hours the solution was decolorized with activated charcoal and was filtered. A clear solution was obtained and was stored in refrigerator in a dark coloured bottle.

**BIOCHEMICAL REAGENTS**

**Acid ammonium molybdate reagent**

\[
\begin{align*}
\text{Ammonium molybdate} & = 25 \text{ g} \\
10\text{N} \text{ H}_2\text{SO}_4 & = 500 \text{ ml} \\
(137.5 \text{ ml H}_2\text{SO}_4 \text{ in 362.5 ml distilled water})
\end{align*}
\]

These ingredients were mixed and 500 ml of distilled water was added to it.

**Amino-naphthol sulphonic acid (ANSA)**

15 g of sodium/metabisulphite were dissolved in 200 ml of distilled water. To 195 ml of this solution, 0.5 g of 1-amino-2-naphthol-4-sulphonic acid and 5 ml of sodium sulphite (20%) solution was added. This solution was decolorized with 1 g of activated charcoal and was kept in the dark for overnight. The colourless filtrate was stored in the refrigerator.
Biuret reagent

CuSO₄ = 1.5 g
NaK tartarate = 6 g
Distilled water = 500 ml
10% NaOH = 300 ml (30 g/300 ml)

The final volume of solution was made up to 1 litre.

FeCl₃ colouring reagent for cholesterol

To 15 ml of concentrated H₂SO₄ was added 1 ml
ferric chloride solution (10 mg FeCl₃·6H₂O/1 ml glacial
acetic acid) agitating well. The volume was made 100 ml
with conc. H₂SO₄. A clear pale yellow solution was obtained.

Acid phosphatase substrate

424 mg of sodium diethylbarbiturate were dissolved in
20 ml distilled water. To this solution was added 500 mg
of sodium beta-glycerophosphate and 5 ml of 1N acetic acid.
The total volume was made 100 ml with distilled water and
pH was adjusted to 5.2 with 0.1N acetic acid or 0.1N NaOH
and was kept in the refrigerator.

Alkaline phosphatase substrate

Dissolved 2.5 g sodium beta-glycerophosphate and
2.12 g sodium diethylbarbiturate in 500 ml of distilled
water. The pH of the solution was adjusted to 8.5 and was stored in the refrigerator.

\[ 1N \text{ CH}_3\text{COOH} \]

To 57.7 ml glacial acetic acid was added 942.3 ml distilled water.

**Sodium periodate solution (0.2 M)**

4.28 g of sodium periodate (meta) was dissolved in 9 M phosphoric acid and final volume was made 100 ml.

**9 M Phosphoric acid**

88.2 ml of phosphoric acid was taken and volume was made 100 ml with distilled water.

**Sodium arsenite solution**

10 g of sodium arsenite was dissolved in 100 ml of 0.5 M sodium sulphate solution prepared in 0.1N H\text{2}SO\text{4} (0.28 ml conc. H\text{2}SO\text{4} was taken and volume was made 100 ml with distilled water).

**0.5 M Sodium sulphate solution**

7.1 g of sodium sulphate was dissolved in 0.1N H\text{2}SO\text{4} and final volume was made 100 ml.
Thiobarbituric acid

600 mg of thiobarbituric acid was dissolved in 0.5 M sodium sulphate solution.

Dinitrophenylhydrazine reagent for ascorbic acid

The reagent was prepared by adding:

2,4-dinitrophenyl hydrazine = 2 g
Thiourea = 250 mg
Copper sulphate = 30 mg

These ingredients were dissolved in 100 ml of 9N H$_2$SO$_4$.

65% H$_2$SO$_4$

To 100 ml of concentrated H$_2$SO$_4$ was added 63.32 ml distilled water.

Potassium bromide-bromine reagent

100 ml water was saturated with bromine and 12 g of potassium bromide was dissolved in it.

Thiourea solution

2 g of sodium tetraborate was dissolved in 100 ml of 4% thiourea solution.
0.25 M Sucrose solution

6.4 g of sucrose was dissolved in 100 ml distilled water.

0.1 M phosphate buffer (0.1 M, pH 7.4)

Stock solution

A: 0.2 M monobasic sodium phosphate ($\text{Na}_2\text{HPO}_4.\text{H}_2\text{O}$)
- 2.76 g in 100 ml of distilled water.

B: 0.2 M dibasic sodium phosphate
- 5.36 g of $\text{Na}_2\text{HPO}_4.7\text{H}_2\text{O}$ or 7.6 g of $\text{Na}_2\text{HPO}_4.12\text{H}_2\text{O}$
100 ml of distilled water.

For pH 7.4: 19 ml of A and 81 ml of B were mixed and volume was made to 200 ml with distilled water.

Glycine buffer (0.1 M)

Glycine = 7.505 g
Sodium chloride = 5.85 g

The above ingredients were dissolved in 100 ml distilled water and the final volume was made to 1 litre with distilled water.
Buffered substrate for LDH

The substrate was prepared by mixing:

Glycine buffer = 125 ml
0.1 N NaOH = 75 ml
Sodium lactate solution (70%) = 5 ml

Citrate buffer (0.1M, pH 6.2 and 0.067M, pH 6.0)

A = 0.1 M solution of citric acid (2.101 g in 100 ml of distilled water)

B = 0.1 M solution of citrate (2.941 g C₆H₅O₇Na₃·2H₂O in 100 ml distilled water)

For pH 6.2: 7.2 ml of A and 42.8 ml of B were mixed and volume was made to 100 ml with distilled water.

For 0.067 M, pH 6.0: 9.5 ml of A and 41.5 ml of B were mixed and volume was made to 100 ml with distilled water. 67 ml of this was diluted to 100 ml with distilled water.

Tris buffer (0.1 M, 0.2 M, pH 7.6 and pH 9.0)

A = 0.2 M solution of tris
(24.2 g Tris in 1000 ml distilled water)

B = 0.2 M HCl (7.294 ml HCl in 100 ml water).
For 0.2 M pH 7.6: 50 ml of A and 38.4 ml of B were mixed and volume was made to 200 ml with distilled water.

For pH 9.0: 50 ml of A and 5 ml of B were mixed and volume was made to 200 ml with distilled water.

**Alcoholic resorcinol**

1 g thiourea and 250 mg of resorcinol was dissolved in 100 ml acetic acid.

**30% HCl**

To 100 ml of conc. HCl, 21.33 ml of distilled water was added.

**Boric acid – NaOH buffer (0.05 M, pH 9.5)**

1.545 g Boric acid was dissolved in 500 ml distilled water (0.05 M) and the pH was adjusted with NaOH (0.05 M) (200 mg NaOH in 100 ml distilled water).

**DNSA**

Dissolved 1 gm of DNSA in 30 ml of distilled water. To this, 40 ml of 1N NaOH and 30 gm of NaK tartarate was added and finally volume was made to 100 ml.
**ATP-Mg mixture**

93.4 mg of sodium salt of ATP (0.075 M) and 16.2 mg of MgCl₂ (0.04 M) was dissolved in 2 ml of distilled water.

**Histidine, Tris-EDTA buffer**

To 5 ml of distilled water, 77.5 mg of histidine (0.1 M), 60.5 mg of Tris (0.1 M), 18.6 mg of EDTA (0.01 M) and 10.1 mg of MgCl₂ (0.01 M) was dissolved.

**Alkaline copper reagent**

Solution A: 5% copper sulphate

Solution B: 70 grams of sodium carbonate, 22 g of sodium bicarbonate and 26 g of sodium tartarate were dissolved in water and the volume made up to one litre with an excess of water.

One part of A and nine parts of B were mixed before use.

**Tungstic acid**

a) Sulphuric acid (0.15 N): 4.17 ml of concentrated sulphuric acid was diluted to one litre with water.
b) Sodium tungstate (2.2%) • 22 g of sodium tungstate was dissolved in water and made up to one litre. Equal parts of (a) and (b) were mixed immediately before use.

**Phosphomolybdic acid**

a) Brominated sodium molybdate • 300 g of sodium molybdate were mixed in 500 ml of water. To this solution were added two drops of bromine and the final volume made up to one litre with water.

b) Sulphuric acid (25%) • 135 ml of concentrated sulphuric acid was added to 500 ml of water and the volume made up to one litre with more water to get 25% sulphuric acid (by weight) solution.

In a one litre volumetric flask, 500 ml of clear supernatant of (a) and 225 ml of 85% phosphoric acid and 150 ml of (b) were added. The bromine was removed from the solution by blowing air into it and 75 ml of glacial acid was added. The volume was made up to one litre with water.

**Sulphanilic acid solution (0.1%)**

One gram of sulphanilic acid was diluted to one litre in 0.18 N hydrochloric acid.
**Diazotizing reagent**

0.3 ml of the 0.5% sodium nitrite solution was added to 10 ml of the 0.1% sulphanilic acid solution. The solution was made 30 minutes before use.

**Bloor's reagent**

95% ethyl alcohol and diethyl ether were mixed in a ratio of 3:1.

**Biuret reagent for serum proteins (Full strength and half strength)**

A fresh solution of Biuret reagent was prepared everytime by adding 5 parts of 25% sodium hydroxide to one part of 2% copper sulphate. This gave full strength Biuret. For half strength, 25 ml of this was made upto 50 ml in a volumetric flask with water.

**SGOT buffer**

In a 100 ml volumetric flask, was added 29.2mg of alpha ketoglutaric acid and 2.66 g of dl aspartic acid. To these was added a small quantity of 1N sodium hydroxide solution to dissolve them and the pH was adjusted to 7.4. The volume was made upto 100 ml by adding pH 7.4 phosphate
buffer. One drop of chloroform was added as a preservative. The buffer was refrigerated until used.

**SGPT buffer**

In a 100 ml volumetric flask was added 29.2 mg of alpha ketoglutaric acid and 17.8 g of dl alanine. The ingredients were dissolved in a small amount of 1 N sodium hydroxide solution and the pH adjusted to 7.4. The volume was made up to 100 ml with pH 7.4 phosphate buffer and one drop of chloroform was added as a preservative. The buffer was stored in the refrigerator until used.

**2,4 Dinitrophenyl hydrazine reagent for transaminases**

198 mg of dinitrophenyl hydrazine was dissolved and the volume made up to one litre with 1 N HCl.

**2/3 N sulphuric acid**

18.5 ml of concentrated sulphuric acid was added to water and the volume made up to one litre.

**Warren's Formula**

\[ 0.0 \times 0.07 \times 50 = \mu \text{ mole/g} \]
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**Units for weights and concentration**

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