APPENDIX I
This appendix includes the different fixatives, stains and other solutions employed during the histological, histopathological and biochemical analyses during the present course of investigation. Single asterisk (•) marked the solution or stains have been used in the histological and histopathological parts of this study while double asterisk (••) includes solutions employed during biochemical estimations of the different metabolites.

Fixatives and solutions used in Histological and Histopathological preparations:

Bouin's fixative: Bouin's fixative was prepared by mixing
75 ml of saturated aqueous picric acid, 25 ml of 40% formalin and 5 ml of glacial acetic acid.

Alcoholic Bouin's fixative: - 1 gm of picric acid crystals were dissolved in 60 ml of 40% formalin and 15 ml of glacial acetic acid. To this was added 150 ml of 80% alcohol and thoroughly shaken.

Zenker's fixative: - 5 gm of mercuric chloride was dissolved in 95 ml of distilled water. 2.5 gm of potassium dichromate was added to it followed by 1 gm of sodium sulphate. The mixture was thoroughly shaken. 5 ml of glacial acetic acid was added before use.

Haematoxylin (Delafield's): - 3.5 gm of haematoxylin was mixed with 100 ml of ethanol and filtered. In another flask, 25 gm of ammonia alum was dissolved in 400 ml of distilled water. To 100 ml of alcoholic haematoxylin, 320 ml of ammonia alum solution was added followed by 80 ml of glycerine. Mixture was shaken vigorously and left as such for a month or so, so as to ensure its complete ripening.

Mallory's Phosphotungstic Acid Haematoxylin (pTAH): - 1.0 gm of haematoxylin and 20 gm of phosphotungstic acid were dissolved
separately in distilled water with gentle heating and were combined when cooled. Total volume was made to one litre with distilled water. For spontaneous ripening, 0.177 gm of potassium permanganate was added to it.

**Alcoholic eosin:** 1.0 gm of eosin was dissolved in 100 ml of 90% alcohol.

**Alcoholic iodine:** Alcoholic iodine solution was prepared by dissolving 6.0 gm of iodine and 4.0 gm of potassium iodide in 100 ml of distilled water. 10 ml of absolute alcohol was added to it.

**Solutions used in Biochemical Estimations:**

**Anthrone Reagent:** 20 mg of anthrone is dissolved in 100 ml of 80% (V/V) sulphuric acid. Every time 1 ml of HCl and 0.1 ml of 90% formic acid was added before using. 8.0 ml of anthrone reagent was used for 1.0 ml of glycogen extract.

**Reaction medium for glycogen phosphorylase:** 4 mg of glycogen and 0.57 mg of 5' AMP/ml were dissolved in 0.05M phosphate buffer pH 6.1 containing 0.1M NaF (104.975 mg in 25 ml of buffer).
Enzyme solution for glycogen phosphorylase:

i) Glucose oxidase stock solution (10 mg glucose oxidase/ml of 0.5M phosphate buffer pH 7.0) store at -20°C.

ii) Horseradish peroxidase stock solution (1 mg of horseradish peroxidase (Sigma)/2.0 ml of 0.5M phosphate buffer, pH 7.0). Store at -20°C.

iii) O-Dianisidine stock solution (5 mg/ml methanol) store at 4°C and prepared fresh every week. Just before use mixed 1 ml of glucose oxidase, 1 ml of peroxidase, 0.1 ml of O-dianisidine stock solutions and 7.9 ml of 0.5M phosphate buffer, pH 7.0.

Substrate medium for glycogen phosphorylase (glycogen synthesis):
Prepared a mixture of 60 mM glucose-1 phosphate, 1% glycogen 2 mM 5'-adenylic acid and 0.1% NaF in phosphate buffer pH 6.7. Stored at -20°C.

0.03% Cysteine: Prepared fresh for each use from 0.3% cystein hydrochloride neutralized to pH 6.7.

Complete medium: Just before use, mix equal volumes of substrate medium and 0.03% cystein.

Reducing reagent: 95% NaHSO₃, 4.5% Na₂SO₃ and 0.5% ANSA.
Phosphate reagent: - Freshly mixed 1 ml of 2.5% ammonium molybdate, 7.0 ml of distilled water, 0.02 ml of 1% copper sulphate, and 1.0 ml of fresh reducing reagent.

Buffered substrate for phosphohexose isomerase

0.0024% G-6-P: - Dissolved 42.5 mg of G-6-P Na₂ salt and 6.1 g of tris in 300 ml of distilled water. Added 125 ml of 0.1M HCl and adjusted the pH to 7.4. Diluted the above with distilled water to 500 ml. (This solution is stable for 6 months at 5°C).

Sodium chloride 0.1%: - Dissolved 9.0 g of sodium chloride in 1 litre of distilled water.

Resorcinol 0.1%: - Dissolved 100 mg resorcinol in 100 ml ethanol (95%). Stored in amber bottle at 5°C.

Hydrochloric acid 10%: - Carefully added 250 ml of concentrated HCl to 50 ml of distilled water and mixed well.

0.31M TCA: - Dissolved 10 g of TCA in 150 ml of distilled water and made the volume to 200 ml.

Reagents for Glucose-6-Phosphatase:

Citrate buffer (0.1M, pH 6.5): - Dissolved 2.101 gm of citric
acid in 50 to 75 ml of distilled water. Adjusted the pH to 6.5 with 30% (w/v) NaOH or KOH and diluted to 100 ml with distilled water.

**Glucose-6-phosphate (0.08M, pH 6.5):** 417 mg of glucose-6-phosphate (barium salt 7H₂O) was dissolved in 2-3 ml of distilled water by the addition of minimum amount of 1N HCl. Added 114 mg of Na₂SO₄ or 139 mg of K₂SO₄ and mixed thoroughly. The contents were centrifuged and the precipitate of barium sulphate was discarded. Carefully a drop of Na₂SO₄ solution was added to the supernatant. When no precipitate was formed the pH was adjusted to 6.5 with 30% (w/v) KOH or NaOH and diluted to 10 ml with distilled water.

**Ammonium molybdate (2 x 10⁻³M):** Dissolved 2.5 gm of ammonium molybdate in 500 ml distilled water. Carefully added 14 ml of concentrated H₂SO₄ to 200 ml of distilled water. Poured the dilute acid into molybdate solution and made the volume to 1000 ml with distilled water.

**Reducing reagent (4.2 x 10⁻²M):** Dissolved 5.7 gm of NaHSO₃ and 0.2 gm of Na₂SO₃ in 50 ml distilled water. Dissolved 0.1 gm amino-napthol sulphonic acid (ANSA) in this mixture and made the final volume to 100 ml with distilled water.
**Substrate solution for acid phosphatase:**

- Sodium β-glycerophosphate \(1.25\) \(\text{gm}\)
- Sodium barbitone \(1.06\) \(\text{gm}\)
- Acetic acid (1N) \(25\) \(\text{ml}\)

Total volume was increased to \(250\) \(\text{ml}\) and pH adjusted at \(4.9\).

**Substrate solution for alkaline phosphatase:**

- Sodium β-glycerophosphate \(2.5\) \(\text{gm}\)
- Sodium barbitone \(2.12\) \(\text{gm}\)

Total volume was increased to \(500\) \(\text{ml}\) and pH was adjusted at \(8.9\).

**Acid ammonium molybdate solution:**

- Ammonium molybdate \(25\) \(\text{gm}\)
- 10N sulphuric acid \(500\) \(\text{ml}\)

(137.5 \(\text{ml}\) sulphuric acid diluted to \(500\) \(\text{ml}\) with distilled water).

The above ingredients were dissolved and the volume made to \(1000\) \(\text{ml}\).

**Amino naphthol sulphonic acid (ANS):**- To \(200\) \(\text{ml}\) distilled water were added \(12\) \(\text{gm}\) of sodium metabisulphite, \(2.4\) \(\text{gm}\) sodium sulphate and \(0.2\) \(\text{gm}\) amino naphthol sulphonic acid powder. The solution was decolorised with activated charcoal and filtered.
Substrate buffer solution for glutamate oxalacetate transaminase (GOT) (0.1M phosphate buffer, pH 7.4, 0.1M L-aspartate, $2 \times 10^{-3} \text{M } \alpha$-oxoglutarate): - Dissolved 1.5 gm $\text{K}_2\text{HPO}_4$, 0.2 gm $\text{KH}_2\text{PO}_4$, 0.039 gm Na-$\alpha$-oxoglutarate and 1.57 gm Na-L-aspartate in 100 ml double glass distilled water. Adjusted pH to 7.4 with 0.4N NaOH.

Substrate solution for glutamate pyruvate transaminase (GPT) (0.1M phosphate buffer pH 7.4, 0.2M DL-alanine, $2 \times 10^{-3} \text{M } \alpha$-oxoglutaric acid): - Dissolved 1.50 gm $\text{K}_2\text{HPO}_4$, 0.20 gm $\text{KH}_2\text{PO}_4$, 0.030 gm $\alpha$-oxoglutaric acid and 1.78 gm DL-alanine in double glass distilled water to make the volume upto 100 ml. Checked the pH with glass electrode.

Ketone reagent ($10^{-3} \text{M}, 2,4 \text{ DNP})$: - 20 mg of 2,4 dinitrophenylhydrazine was dissolved in 1N HCl and volume was made upto 100 ml.

Sodium hydroxide (0.4N): - 16 gm sodium hydroxide pellets were dissolved in 500 ml of distilled water. After thorough mixing, the volume was increased to one litre.

Sodium pyruvate ($2 \times 10^{-3} \text{M}$): - 22 mg of sodium pyruvate were dissolved in 50-60 ml of double glass distilled water and volume increased to 100 ml.