APPENDIX
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The reagents for various biochemical determinations were prepared according to the following procedure.

1. Reagents for Nitrogen estimation

1.1 Nessler's Reagent:–

10 g of potassium iodide was dissolved in 10 ml of distilled water. To this was added a solution of mercuric chloride (6 g in 100 ml of water) in small lots and with shaking till a slight permanent precipitate was formed. To this was added 80 ml of 9 N potassium hydroxide solution and then diluted to 200 ml with distilled water. The solution was kept overnight and the clear solution decanted for use.

2. Reagents for Phosphorus estimation

2.1 Molybdate Reagent:–

6.25 g of ammonium molybdate was dissolved in 75 ml of 10N H_{2}SO_{4}. To this solution 175 ml of distilled water was added in order to get 250 ml of the above reagent.

2.2 Amino naphthol sulphonic acid:–

0.5 g of i, 2, 4, Amino-naphthol-sulphonic acid was dissolved in 195 ml of 15% sodium bisulphite solution to which 5 ml of 20% sodium sulphite solution was added. The above solution was stored in a dark coloured bottle.

3. Reagents for Protein estimation

3.1 Reagent A:–

2% Sodium carbonate was mixed with 0.1N sodium hydroxide solution.
3.2. **Reagent B**:

0.5% Copper sulphate (CuSO$_4 \cdot 5$H$_2$O) was added to 1% potassium sodium tartrate.

3.3. **Reagent C (Alkaline Copper sulphate solution)**:

It was prepared by mixing 50 ml of reagent ‘A’ and 1 ml of reagent ‘B’ prior to use.

3.4. **Reagent D (Folin-Ciocalteau reagent)**:

100 g sodium tungstate (Na$_2$WO$_4 \cdot 2$H$_2$O) and 25 g sodium molybdate (Na$_2$MoO$_4 \cdot 2$H$_2$O) was dissolved in 700 ml distilled water in which 50 ml of 85% phosphoric acid and 100 ml of concentrated hydrochloric acid was mixed. The flask was connected with a reflux condenser and boiled gently on a heating mantle for 10 h. At the end of the boiling period, 150 g lithium sulphate, 50 ml distilled water and 3-4 drops of liquid bromine was added to this flask. The reflux condenser was removed and the solution in the flask was boiled for 15 minutes in order to remove excess bromine, cooled and diluted to 1 liter. The strength of this acidic solution (1 N) was tested by treating it with 1 N sodium hydroxide using phenolphthalein as an indicator.

4. **Reagent for Calcium and Sodium estimation**

4.1 **TAM solution (Tri acid mixture)**

It is a mixture of three acids like nitric acid, sulphuric acid and per chloric acid in the ratio of 10:5:4.