APPENDIX
PREPARATION OF REAGENTS

The various reagents used for biochemical determinations were prepared according to the following methods.

1. REAGENTS FOR THE ESTIMATION OF PYRIDOXINE

1.1 Chloroimide reagent

100 mg crystalline 2,6-dichloroquinone chloroimide was dissolved in 250 ml isopropanol. The solution was stored in a glass-stoppered bottle in refrigerator and discarded if pink colour developed.

1.2 Ammonia-ammonium chloride solution

160 mg ammonium chloride was dissolved in 70 ml distilled water to which 160 ml ammonia-water (27% approximately) was added. The solution was diluted upto 1 l with distilled water.

1.3 Boric acid solution (5%)

5 g boric acid was dissolved in distilled water and the final volume was made upto 100 ml.

1.4 Pyridoxine hydrochloride solution

100 mg pyridoxine hydrochloride was dissolved in sufficient distilled water and the final volume was made upto 1.1 The solution was stored in an amber coloured bottle in refrigerator.
1.5 Buffer solution (pH 3)

73 g sodium phosphate dihydrate and 167 g citric acid was dissolved in sufficient distilled water and the final volume was made up to 1 l.

2. REAGENTS FOR THE ESTIMATION OF NITRATE REDUCTASE ACTIVITY

2.1 Phosphate buffer (pH 7.5)

(a) 13.6 g potassium dihydrogen orthophosphate (KH$_2$PO$_4$) was dissolved in sufficient distilled water and the final volume was adjusted up to 1 l.

(b) 17.42 g dipotassium monohydrogen orthophosphate (K$_2$HPO$_4$) was dissolved in sufficient distilled water and the final volume was made up to 1 l.

(c) 160 ml of solution (a) and 840 ml of solution (b) was mixed in order to get pH 7.5.

2.2 Potassium nitrate (0.2M)

2.02 g potassium nitrate was dissolved in enough distilled water and the final volume was made up to 100 ml.

2.3 Sulphanilamide solution (1%)

1 g sulphanilamide powder was dissolved on 100 ml 3H HCl.

2.4 NED-HCl solution (0.02%)

20 mg NED-HCl N-1(naphthyl)-ethylene diamine dihydrochloric acid was dissolved in 100 ml distilled water.
3. REAGENTS FOR THE DETERMINATION OF N AND P

3.1 Nessler's reagent

(a) 3.5 g potassium iodide was dissolved in 100 ml distilled water to which 4% mercuric chloride was mixed with continued stirring till a slight red precipitate remained (about 325 ml of the solution was required).

(b) 120 g of sodium hydroxide was dissolved in distilled water and final volume made upto 250 ml.

(c) Solutions (a) and (b) were mixed together and diluted to 1 l with distilled water. The solution was stored in an amber coloured bottle in refrigerator.

3.2 Molybdic acid reagent (2.5%)

6.25 g ammonium molybdate was dissolved in 75 ml 10N H₂SO₄. To this solution, 175 ml distilled water was added in order to get 250 ml of the above reagent.

3.3 Aminonaphthol sulphonic acid

0.5 g 1-amino-2-naphthol-4-sulphonic acid was dissolved in 195 ml 15% sodium bisulphite solution to which 5 ml of 20% sodium sulphite solution was added. The above solution was stored in dark coloured bottle.

4. REAGENTS FOR THE ESTIMATION OF CARBOHYDRATE

4.1 Sulphuric acid (1.5 N)

10.20 ml pure sulphuric acid (AR) was added to
enough distilled water and final volume was made upto 250 ml.

4.2 Phenol (5%)

5 ml distilled phenol was mixed with 95 ml distilled water

5. REAGENTS FOR THE ESTIMATION OF PROTEIN

5.1 Reagent A

2% sodium carbonate was mixed with 0.1N sodium hydroxide (1:1).

5.2 Reagent B

0.5% copper sulphate was added to 1% sodium tartrate (1:1).

5.3 Reagent C (alkaline copper sulphate solution)

It was prepared by mixing 50 ml reagent 'A' with 1 ml reagent 'B'.

5.4 Reagent D (carbonate-copper sulphate solution)

Same as reagent 'C', except for the omission of sodium hydroxide.

5.5 Reagent E (Folin's phenol reagent)

100 g sodium tungstate and 25 g sodium molybdate was dissolved in 700 ml distilled water in which 50 ml of 85% phosphoric acid and 100 ml conc. 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 building 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 min. in order to remove excess bromine, cooled and diluted to 1 l.

The strength of this acidic solution (1 N) was tested by treating it with 1 N sodium hydroxide using phenolphthalein as an indicator.