APPENDIX
PREPARATION OF REAGENTS

The various chemicals/reagents used for biochemical analyses were prepared by adopting the following procedures.

1. **Reagents for the estimation of carbonic anhydrase activity**

1.1 **Cystein hydrochloride solution (0.2 M)**

48 g cystein hydrochloride was dissolved in sufficient quantities of double distilled water (DDW) and final volume was made up to 1000 cm$^3$.

1.2 **Phosphate buffer (pH 6.8)**

(a) 27.8 g sodium dihydrogen orthophosphate (NaH$_2$PO$_4$) was dissolved in sufficient quantities of DDW and the final volume was made up to 1000 cm$^3$.

(b) 53.65 g di-sodium hydrogen orthophosphate (Na$_2$HPO$_4$) was dissolved in sufficient quantities of DDW and the final volume was made up to 1000 cm$^3$.

(c) 51 cm$^3$ of solution (a) and 49 cm$^3$ of solution (b) was mixed and final volume was made up to 200 cm$^3$ with DDW in order to get the required pH 6.8.

1.3 **0.2 M Sodium bicarbonate solution in 0.02 M sodium hydroxide solution**

16.8 g sodium bicarbonate was dissolved in aqueous sodium hydroxide solution (0.8 g NaOH/l) and final volume was made up to 1000 cm$^3$ with sodium hydroxide solution.
1.4 Bromothymol blue (0.002%)

0.002 g bromothymol blue was dissolved in sufficient quantities of ethanol and the final volume was made up to 100 cm³.

1.5 Hydrochloric acid (0.01 N)

0.86 cm³ pure hydrochloric acid was added to enough DDW and final volume was made up to 1000 cm³.

2. Reagents for the estimation of nitrate reductase activity (NRA)

2.1 Phosphate buffer (pH 7.5)

(a) 13.6 g potassium dihydrogen orthophosphate (KH₂PO₄) was dissolved in sufficient quantities of double distilled water (DDW) and the final volume was made up to 1000 cm³.

(b) 17.4 g dipotassium monohydrogen orthophosphate (K₂HPO₄) was dissolved in adequate amount of double distilled water and the final volume was made up to 1000 cm³.

(c) 160 cm³ of solution (a) and 840 cm³ of solution (b) was mixed in order to get the required pH 7.5.

2.2 Potassium nitrate (0.2 M)

2.0 g potassium nitrate was dissolved in required quantity of DDW and the final volume was made up to 100 cm³.

2.3 Isopropanol solution (5%)

5 cm³ isopropanol was added to 95 cm³ of DDW.
2.4 **NED-HCl solution (0.02%)**

20 mg N-1-(naphthyl)-ethylene diamine dihydrochloric acid (NED-HCl) was dissolved in enough DDW and the volume was made up to 100 cm³.

2.5 **Sulphanilamide solution (1%)**

1g sulphanilamide powder was dissolved in 100 cm³ of 3N hydrochloric acid.

2.5.1 **Hydrochloric acid (3N)**

26.2 cm³ pure hydrochloric acid was added to enough DDW and the final volume was made up to 100 cm³.

3.0 **Reagents for the estimation of proteins**

3.1 **Reagent A**

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

3.2 **Reagent B**

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

3.3 **Reagent C (alkaline copper sulphate solution)**

It was prepared by mixing 50 cm³ reagent ‘A’ with 1 cm³ reagent ‘B’.

3.4 **Reagent D (Carbonate copper sulphate solution)**

It was prepared in the same way as reagent ‘C’ except the omission of sodium hydroxide from reagent ‘A’.