PREPARATION OF REAGENTS

The reagents for various biochemical determination were prepared according to the following methods:

1. Reagents for NPK determination

a. Nessler's reagent

3.5 g of potassium iodide was dissolved in 100 ml of distilled water in which 4% mercuric chloride solution was added with stirring until a slight red precipitate remained (about 325 ml of the solution was required). Therefore, 120 ml of sodium hydroxide with 250 ml distilled water were mixed. The volume was made upto 1000 ml with distilled water. The mixture was decanted and kept in amber coloured bottle.

b. Molybdic acid reagent (2.5%)

6.25 g of ammonium molybdate was dissolved in 175 ml distilled water to which 75 ml of 10 N-sulphuric acid was added.

c. Aminonaphthol sulphonic acid

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

2. Reagents for protein estimation

a. Reagent A

0.5% copper sulphate solution and 1% sodium sulphate solution were mixed in equal volumes.

b. Reagent B

50 ml of 2% sodium carbonate solution was mixed with 1 ml of reagent 'A'.

c. Folin's reagent

100 g of sodium tungstate and 25 g of sodium molybdate were dissolved in 700 ml of distilled water to which 50 ml of 85% phosphoric acid and 100 ml of concentrated hydrochloric acid were added. The solution was reflected on a heating mantle for 10 h. At the end, 150 g of lithium sulphate, 50 ml of distilled water and 3-4 drops of liquid bromine were added. The reflex condensor was removed and the solution was boiled for 15 min to remove excess bromine, cooled and diluted upto 1000 ml. The strength of this acidic solution was adjusted to 1 N by titrating it with 1 N-sodium hydroxide solution.