APPENDIX XIX
ESTIMATION OF RETINOL
(Neeld and Pearson, 1963)

Principle

This method is based on the measurement of the unstable blue color formed by the interaction of vitamin A with TFA (Trifluoroacetic acid). The intensity of which is a function of the concentration of vitamin A measured at 620 nm.

Reagents
1. 2 N KOH in 90 per cent ethanol
2. TFA reagent : TFA : chloroform - 1:2 (prepared freshly)
3. Standard vitamin A solution : 34.4 mg of vitamin A acetate or 30 mg of vitamin A dissolved and made upto 100 ml with chloroform. 1 ml of stock contains 300 ug of retinol

a. Estimation of Retinol in liver

Since retinol is light sensitive, all procedures were carried out in a darkened room.

0.5 - 0.1 g of liver was homogenised in distilled water to obtain a 20 per cent homogenate. Equal volume of saponification mixture (2 N KOH in 90 per cent alcohol) was added to the homogenate and heated under gentle reflux for 20 minutes at 60°C. 25 ml of water was added to the mixture
after cooling to room temperature and the solution was transferred to a separating funnel. It was then extracted thrice using 25, 15 and 15 ml of petroleum ether (boiling point range 40°C - 60°C). The ether extracts were pooled and washed with 50-100 ml of distilled water repeatedly until the wash was free of alkali. The petroleum ether extract was then dried by adding anhydrous sodium sulphate. The volume of the extract was noted and aliquotes were taken in triplicates.

The aliquot was evaporated to dryness in a 60°C water bath. The residue was taken immediately in 0.1 ml of chloroform and 0.1 ml of acetic anhydride and 1.0 ml of TFA reagent were added to it. The mixture was rapidly transferred to a cuvette and the absorbance was measured at 620 nm, against a reagent blank, exactly 15 seconds after the addition of the TFA reagent, in a UV - VIS 108 spectro photometer.

Standard curve was made using retinyl acetate solution in chloroform containing concentrations ranging from 0.5 to 2 ug. The results were expressed as mean of ug retinol/g tissue.
b. Estimation of plasma retinol

The micromethod of plasma vitamin A estimation as described by Neeld and Pearson (1963) was adopted to estimate the circulating levels of vitamin A.

0.2 - 0.5 ml plasma was taken in a test tube and an equal volume of 95 per cent ethanol and 0.6 ml petroleum ether were added to it. The tube was corked and the contents agitated in a cyclomixer for 45 seconds to extract the vitamin A into petroleum ether. The tubes were centrifuged. The petroleum ether layer was transferred to a test tube and evaporated to dryness at 60° C. The residue was dissolved in 0.05 ml of chloroform < 0.05 ml acetic anhydride and 0.5 ml of TFA reagent were added to it and absorbance measured at 620 nm exactly 15 seconds after the addition of the reagent, in a uv-vis 108 spectrophotometer.

The standard curve was prepared using 0.1 - 0.5 µg of retinyl acetate in chloroform.

The results are expressed as mean of µg of retinol/100 ml plasma.