II. MATERIALS

**Papain** - Twice crystallized papain (Lot No. 108B-2240) was purchased from the Sigma Chemical Company, U.S.A. as a suspension in 0.05M acetate buffer, pH 4.5. The suspension containing 27 mg of enzyme per ml was centrifuged and dissolved in 0.1M phosphate buffer, pH 7.5. Its protein concentration was determined spectrophotometrically at 280 nm using an $E_{1\text{cm}}^{280}$ value of 25.0 (Mitchel *et al.*, 1970).

**Ficin** - Twice crystallized ficin (Lot No. 96C-9510) was obtained as a suspension in 2.0M NaCl and 0.03M cysteine, pH 5.0 from the Sigma Chemical Company, U.S.A. The pH of the suspension (28 mg enzyme per ml) was raised to 7.5 by adding 0.1M phosphate buffer, pH 7.5 containing 0.001M EDTA. The resulting solution was centrifuged to remove any insoluble material and its protein concentration was determined spectrophotometrically at 280 nm using an $E_{1\text{cm}}^{280}$ value of 21.0 (Englund *et al.*, 1968).

**Trypsinogen** - Crystallized trypsinogen (Lot No. TG 1 DB) was purchased from the Worthington Biochemical Corporation, U.S.A.

**β-Trypsin** - It was isolated from the crystallized trypsin of the Worthington Biochemical Corporation by chromatography on SP-sephadex C-50 at pH 7 in the presence of 0.001M benzamidine hydrochloride according to Schroeder and Shaw (1968).
Azo albumin - Azo albumin (Lot No. 86C-7590) was a product of the Sigma Chemical Company, U.S.A. A solution of 1% azo-albumin in 0.1M phosphate buffer, pH 7.5, was made and stored at 4°C. It was used within a week as a substrate for the determination of proteolytic activity of cysteine proteases (Tomarelle et al., 1949). Prior to use, its concentration in 0.1M NaOH was estimated spectrophotometrically at 440 nm using an E1%1cm value of 36 (Sigma, 1978).

Casein - Casein was prepared from cow milk according to a method described by Dunn (1949). A stock solution of 0.5% or 1% casein was made by suspending 0.5 g or 1 g of casein in 100 ml of 0.1M phosphate buffer, pH 7.5, containing 0.001M EDTA. The suspension was heated for about 15 minutes in a boiling water bath until all the casein was dissolved and then diluted to 100 ml with double distilled water. The solution was kept at 4°C and was used within a week. The casein solution was used as a substrate for determination of proteolytic activity.

Haemoglobin - Haemoglobin was prepared from bovine red blood cells according to Northrop et al. (1943). It was denatured with alkaline urea solution and was used as a substrate for the determination of proteolytic activity of enzymes (Anson, 1939).

Cow 3-lactoglobulin B - Four times crystallized 3-lactoglobulin B was a product of this laboratory prepared by the method of Aschaffenburg and Drewry (1957).
Bovine serum albumin - Crystallized bovine serum albumin (Lot No. 86B-0250) was purchased from the Sigma Chemical Company, U.S.A.

Lysozyme - Hen lysozyme (Lot No. 75B-8830) was purchased from the Sigma Chemical Company, U.S.A.

Ovalbumin - Five times crystallized ovalbumin (Lot No. A 70B-62) was obtained from the Sigma Chemical Company, U.S.A.

Carboxymethyl cellulose (CM-cellulose) - CM-cellulose was purchased from the BioRad Laboratories, U.S.A. Its exchange capacity was 0.74 meq per g. The cellulose was washed successively first with 0.1N NaOH, then with 0.1N HCl and finally with glass distilled water. The suspension in water was stored at 4°.

Sulphopropyl-sephadex (SP-sephadex) C-50 - SP-sephadex C-50 (Lot No. 0381) was purchased from the Pharmacia, Uppsala, Sweden. Its exchange capacity was 2.3 ± 0.3 meq per g and particle size was 40-120μ. The material was washed successively first with 0.1N NaOH, then with 0.1N HCl and finally with glass distilled water. The suspension in water was stored at 4°.

Diethyl aminoethyl-sephadex (DEAE-sephadex) A-25 - DEAE-sephadex A-25 (Lot No. 8813) was purchased from the Pharmacia, Uppsala, Sweden. Its particle size was 40-120μ and exchange capacity was 3.5 ± 0.5 meq per g. It was washed successively first with 0.1N HCl, then with 0.1N NaOH and finally with glass distilled water. The suspension in water was stored at 4°.
Sephadex G-25 - Sephadex G-25 (Lot No. To 4614) was obtained from the Pharmacia, Uppsala, Sweden. It had a water regain of 2.30 per g.

Benzoyl-DL-arginine p-nitroanilide hydrochloride (BAPA) - This was purchased from the Schwarz/Mann, U.S.A.

5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) - This was a product of the Aldrich Chemical Company, Inc., U.S.A.

Acrylamide, N,N'-methylenebisacrylamide, N,N,N',N'-tetra-methylethylenediamine (TEMED) and riboflavin - These were products of Eastman Organic Chemicals, U.S.A. and were used in polyacrylamide gel electrophoresis.

Ethylene diaminetetra acetic acid (EDTA) - Reagent grade EDTA was obtained from the S.M. Chemicals, India.

Glutathione - Reduced glutathione (Lot No. GH 6604) was obtained from the Schwarz Bio Research, Inc., U.S.A.

Cysteine-hydrochloride - This was obtained from the Agro Industries Pvt. Ltd., India.

Cleland's reagent (Dithioerythritol) - Dithioerythritol (Lot No. C/D 73-3) was purchased from the Pierce Chemical Company, U.S.A.

Sodium dodecyl sulphate (SDS) - SDS (Lot No. 196C-0188) was obtained from the Sigma Chemical Company, U.S.A.
Coomassie blue - Coomassie blue (Lot No. 10) was purchased from the Miles Laboratory, U.S.A.

3-alanine - This was obtained from E. Merck, West Germany.

Sodium tetrathionate - Sodium tetrathionate was prepared according to a method described by Gilman et al. (1946) and was stored at 4°C.

Iodoacetic acid - This was recrystallized from chloroform and was stored in the dark at -10°C.

Cellulose casing - Visking seamless cellulose casings were products of the Union Carbide Corporation, U.S.A. Cellulose tubings of 8/32, 20/32- or 36/32-inch inflated diameter, depending on the volume of solution required, were used for dialysis.

All other chemicals were of reagent or analytical grade.