MATERIAL AND METHODS (GENERAL)
Collection and maintenance of scorpions

Scorpions, *Palamneus phipsoni*, were collected around Pune by local field collectors, and kept in cages containing moist soil. They were fed on insects like grasshoppers and cockroaches. Water was sprinkled on the soil periodically to keep it always moist. The scorpions were used in experiments within a week after their collection in the field.

Preparation of tissue extracts

The scorpions were dissected under ether anesthesia. In all enzymatic studies, the tissues excised from the animals were immediately rinsed in ice-cold homogenizing medium, blotted and rapidly weighed on a Mettler balance (Mettler Felikanstrasse, 19 Zurich, Switzerland, Model H 16, precision ± 0.01 mg). Whenever the digestive tract was used in these studies, it was first opened up and then washed thoroughly with ice-cold homogenizing medium to remove the gut contents. Homogenates of the tissues were prepared with an all glass tissue grinder kept in an ice bath. These homogenates were used for enzyme assays after suitable dilution.

The different methods employed in the biochemical and enzymatic analysis of scorpion tissues are described in the respective chapters.
Protein determination

Protein content in the tissue extracts was determined by the method of Lowry, Rosebrough, Farr and Randall (1951) as described by Dairain, Besch, Couri and Goldyn (1966). Tissue homogenates used in enzyme assays were diluted appropriately with distilled water and duplicate aliquots of these were used for protein estimation. Protein standards were prepared from crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri, U.S.A.).

Samples and standards were made up to 1.0 ml with distilled water. They were mixed with 5.0 ml of alkaline copper reagent and allowed to stand for 10 minutes at room temperature. Then 0.5 ml of Folin-Gioacalteu reagent (1.0 N) was added. They were mixed immediately and allowed to stand for 30 minutes at room temperature. The colour intensity of the samples was measured in Klett-Summerson photoelectric colorimeter employing filter No. 69 (transmission 660–740 nm).

Chemicals

Chemicals used were reagent grade products of either British Drug Houses, E. Merck, Fluka or Sarabhai Merck. The sources of other important biochemicals used in the present study are given in the respective chapters.