Summary

Fruton and collaborators[16, 54, 55] discovered an intracellular endopeptidase in beef spleen which appeared homospecific with chymotrypsin when acting on dipeptide amides or esters. According to Fruton this enzyme (named cathepsin C) may have a function in protein breakdown *in vivo*. This author also suggested a possible involvement of this enzyme in protein or peptide synthesis by virtue of its ability to catalyze transamidation reactions. The studies described in this thesis had their origin in these concepts.

1. Intracellular protein catabolism and cathepsins.

A critical analysis of the relevant literature led us to the conclusion that breakdown of intracellular protein within intact cells is, in contrast to the opinion of some authors, a reality. The mechanism of this phase of protein metabolism is, however, still completely unknown. Knowledge of the enzyme system concerned is a prerequisite for its elucidation.
The literature on the intracellular proteases (cathepsins) which are present in a great diversity of animal tissues is reviewed. Proteolytic activity at pH 3–4 has been demonstrated in all tissues investigated. However, cathepsins active at neutral pH have only been detected in a very limited number of tissues. These "neutral" enzymes are presumably concerned with protein breakdown *in vivo* in view of their activity under physiological conditions. Cathepsin C belongs to this class of enzymes.

2. Enzyme preparation; analytical methods.

A partially purified preparation of cathepsin C was obtained according to de la Haba *et al.*[67] with some minor modifications.
The commonly used methods of assay are discussed and a new method for cathepsin C estimation, using the substrate glycyl-phenylalanyl-p-nitroanilide is described[118]. This method appears to be convenient and rapid, and is especially suitable for the analysis of large numbers of fractions.

3. CHROMATOGRAPHY OF CATHEPSIN C.

Chromatography of proteins and its special problems are discussed. An apparatus for the continuous registration of the optical density at 280 mμ of the column eluate is described.

Cathepsin C, a rather acidic protein, could be further purified by use of anion-exchange columns. The highest degree of purification was achieved by chromatography on a combined column of DEAE-cellulose and DEAE-Sephadex (spec. act. 105 units per mg of protein). Complications arising from the oxidation of the reactive thiol group in the enzyme during chromatography were obviated by the addition of thiols, e.g. cysteine or mercaptoethanol to the eluents, which resulted in an improved elution pattern[119].

According to the sedimentation analysis the purest preparation obtained contained 60 % cathepsin C and 40 % of a much lighter component. The molecular weight of cathepsin C was estimated to be 210,000, in agreement with the value found by de la Haba *et al.* for a much less purified preparation.

4. HYDROLYTIC REACTIONS.

Using the rather unselective methods of Anson and Kunitz no action of cathepsin C on various proteins could be detected. Employing the polypeptide hormones glucagon and β-corticotropin it was shown that the activity of cathepsin C is restricted to the removal of N-terminal dipeptides which have to satisfy narrow specificity requirements[120]. This remarkable specificity was further established with synthetic peptides. The participation of cathepsin C in intracellular protein breakdown must, therefore, be excluded. The results obtained by Fruton *et al.* leading to the conclusion of such a possible role were shown to be due to an other enzyme.

5. TRANSAMIDATION REACTIONS.

In view of the extreme specificity of cathepsin C its ability to catalyze transamidation reactions might well be its physiologically important property. The enzyme was found to possess, besides its high donor specificity, some acceptor specificity, at least regarding the N-terminal amino acid. These reactions will have to be studied further before their possible metabolic significance can be judged.

Cathepsin C must have quite a complex active centre that may possess interesting structural features.