Summary and general conclusions

This thesis deals with structural features determining endocytosis of some proteins by sinusoidal rat liver cells in vivo. An introduction (Chapter 1) shows that binding to the cell surface of endocytosing cells may well be the rate-controlling and discriminating step in the breakdown of circulating proteins. Such a mechanism has been shown to operate in the clearance of glycoproteins, antigen-antibody complexes and low-density lipoproteins.
In the work described in the following chapters, we have studied endocytosis of chemical modifications of bovine pancreatic ribonuclease A and hen egg-white lysozyme. These proteins are not glycosylated or otherwise modified, and they may well be representative for tissue-derived enzymes.
Cross-linking of ribonuclease or lysozyme with the bifunctional reagent dimethyl suberimidate enabled us to study a possible relation between uptake and molecular size (Chapter 2 and Chapter 4). This modification does not change the charge of the protein. The retention of enzyme activity, and of the stability of disulfide bonds towards reduction, as well as the persistence of stability towards endoproteases proved that the cross-linking procedure did not result in gross conformational changes of the ribonuclease and lysozyme molecules. Our results demonstrate that the rate of uptake of the ribonuclease and lysozyme derivatives by liver (and spleen) increases with molecular size, and that liver takes a major share in the clearance from the circulation. Autoradiography, cell separation, and subcellular fractionation of liver showed that the radioactive proteins were eventually taken up into lysosomes of sinusoidal cells.
The correlation between number of protein units in the oligomers and the rate of uptake must be due to increased affinity caused by multiple binding of signal groups on the protein to several receptors on the sinusoidal cell membrane; the dissociation constant of the oligomer will be much lower than that of the corresponding monomer.
We have investigated the chemical nature of these signals on our protein molecules, and found that sinusoidal rat liver cells recognize basic groups. Evidence for the involvement of basic groups was obtained in two ways, as discussed in Chapter 3 and 4. Firstly, we changed the positive charge on the amino groups of ribonuclease and lysozyme to neutral or negative by acetylation or succinylation. The modified proteins did not contain easily reducible disulfide bonds and they were not very susceptible to endoproteases: apparently, the acylation procedures had not caused gross denaturation. The rate of uptake of the acetylated and succinylated proteins by liver and spleen was much less than that of the unmodified species.

The clearance from plasma was also strongly reduced. Secondly, a positive correlation was found between binding of different groups within the ribonuclease and lysozyme derivatives to a cation exchanger and the rate of endocytosis by sinusoidal liver cells.
Thus, uptake of proteins by sinusoidal liver cells is related to the positive charge of the molecules. The positive charge depends on the net number of positively charged groups per monomer as well as on the degree of polymerization.
Adsorptive endocytosis is not the only route by which proteins can enter the lysosomal system. Many cell types interiorize extracellular fluid by a process that is called fluid-phase endocytosis. In this way, substances are taken up non-specifically, as solutes of the extracellular fluid. We have estimated the magnitude of this process by measuring the uptake of labelled polyvinylpyrrolidone by liver cells in vivo (Chapter 5). Polyvinylpyrrolidone is a water-soluble, synthetic polymer; several lines of evidence suggest that it does not adsorb to the cell membrane of liver cells.
Our results show that fluid endocytosis by liver is a relatively slow process which hardly contributes to the clearance of proteins like the derivatives of lysozyme and ribonuclease discussed above. Fluid-phase endocytosis may, however, be significant in the clearance of proteins, e.g. serum albumin, that are not bound to plasma membranes of endocytosing cells. Among other rat tissues, only spleen and lymph nodes show higher rates of fluid endocytosis per weight than liver. Within the liver, fluid endocytosis (again expressed per weight) of sinusoidal cells is about tenfold that by hepatocytes; if the results are expressed per plasma membrane surface, the ratio is about two.

Our results show that derivatives of ribonuclease and lysozyme are rapidly cleared from the circulation because of the adherence of positive groups on the surface of the proteins to the plasma membranes of endocytosing cells. Similar results have been reported for the most basic isoenzyme of lactate dehydrogenase. Assuming that this is a general mechanism for clearance of classes of proteins, one can predict that plasma concentrations of proteins with an abundance of positively charged groups will generally be low. Indeed, plasma contains far fewer basic proteins than most other mammalian tissues. Positive charge may, therefore, be one of the factors controlling the plasma levels of tissue-derived enzymes.
The preferential uptake of positively charged proteins by sinusoidal liver cells cannot simply be attributed to the negative surface charge of these cells, as probably all vertebrate cell surfaces are negatively charged. A partial explanation may be that positively charged proteins bind equally well to the cell membranes of all sorts of cells, but that in sinusoidal liver cells (and spleen cells) the cell membrane is exceptionally rapidly interiorized leading to uptake of the attached proteins. An indication in that

direction is given by the results obtained with polyvinylpyrrolidone. In addition (or alternatively), the cell membrane of sinusoidal liver cells may contain sites with a relatively high concentration of negatively charged groups that bind basic proteins very effectively.
Finally, the quantitative contribution of liver to the clearance of circulating proteins should be considered. Liver is pre-eminent in the selective uptake of glycoproteins, several protein complexes, lactate dehydrogenase isoenzyme M4, some modified forms of albumin, and — as shown in this thesis — native and modified forms of ribonuclease and lysozyme, but also in the non-selective uptake of, for example, albumin by fluid-phase endocytosis. Parenchymal and non-parenchymal liver cells perform different tasks in the selective endocytosis of (classes of) proteins: parenchymal cells contain receptors for uptake of galactose-terminal glycoproteins, and non-parenchymal cells endocytose selectively N-acetylglucosamine- and mannose-terminal glycoproteins, antigen-antibody complexes, and positively charged proteins. Future research may reveal receptors for other (classes of) proteins, as well as the differential role of the individual cell types that constitute the non-parenchymal cell-fraction (like Kupffer cells and endothelial cells).