TABLE OF CONTENTS

LIST OF TABLES....1

LIST OF FIGURES....2

ABBREVIATIONS....3

CHAPTER 1. INTRODUCTION
1.1 Endocytosis and endocytic vesicles....6
1.2 The endocytic process: molecular components and mechanism....6
1.2.1 Molecular components of endosomes and their functions....6
1.2.1.1 The endosomal proteins....7
1.2.1.1.1 Proteins specific to coat formation....7
1.2.1.1.2 Proteins specific to fission machinery....9
1.2.1.1.3 The SNARE(s) family....9
1.2.1.1.4 The Ras GTPase family....10
1.2.1.1.5 LAMP....11
1.2.1.1.6 Vacuolar H^+-ATpase(s)....12
1.2.1.1.7 Actin....12
1.2.1.2 The endosomal proteases....12
1.2.1.3 The endosomal lipids....13
1.2.2 Mechanism of endocytosis....15
1.2.2.1 Types of endocytosis and endocytic vesicles....15
1.2.2.1.1 Phagocytosis....15
1.2.2.1.2 Pinocytosis....16
1.2.3 Models for endocytosis....21
1.2.3.1 Vesicle shuttle model....22
1.2.3.2 Maturation model....22
1.3 Significance of endocytosis in cell biology....23
1.3.1 Functions of endocytic vesicles....23
1.3.2 Significance in pathophysiology....24
1.3.2.1 Microbial pathogenesis....24
1.3.2.2 Genetically predisposed disorders....25
1.3.3 Role of endocytosis in drug delivery....26
1.4 Tools for analysis of endocytosis....27
1.4.1 Tools for endosome isolation/purification....27
1.4.1.1 Centrifugation methods....27
1.4.1.2 Free flow electrophoresis....28
1.4.1.3 Magnetic affinity chromatography....28
1.4.2 Tools for endosome characterization to study endocytic reactions....29
1.4.2.1 Kinetic studies....29
1.4.2.2 *In vitro* assays....29
1.4.2.3 Immunological tools....30
1.4.2.4 Genetic tools and mutant analysis....30
1.4.2.5 Microscopic tools....30
1.5 Project aims....31
1.5.1 Objectives....31

CHAPTER 2. MATERIALS AND METHODS
2.1 Animal usage, primary cell culture and cell lines 35
2.1.1 Animal protocol 35
2.1.2 Isolation of rat peritoneal macrophages 35
2.1.3 Maintenance of cell lines 36
2.2 Isolation of endosomes 36
2.2.1 Endocytic probes 36
2.2.2 Fluid-phase pinocytosis of endosomal probes 37
2.2.3 Subcellular fractionation 39
2.2.4 Electromagnetic purification of endosomes 40
2.3 Characterization of endosomes 40
2.3.1 Protein and lipid quantitation 40
2.3.2 Fluorescence analysis 41
2.3.3 Analysis of intravesicular pH of endosomes 41
2.3.4 *In vitro* acidification of endosomes 42
2.3.5 Biochemical characterization of isolated endosomes 43
2.3.6 Fluorescence microscopy of endosomes 44
2.3.7 Electron microscopy of endosomes 45
2.3.8 Confocal microscopy 45
2.3.9 Analysis of endosomal proteins 46
2.3.10 Analysis of endosomal lipids 50

CHAPTER 3. RESULTS
3.1 Development of probes and establishment of conditions for endocytosis 52
3.1.1 Preparation of iron-dextran 52
3.1.2 Viability of iron-dextran and FITC-dextran fed rat peritoneal macrophages 52
3.1.3 Disruption of rat peritoneal macrophages 53
3.1.4 Fluid-phase endocytosis of endosomal probes 54
3.2 Purification and characterization of endosomes 54
3.2.1 Electromagnetic affinity chromatography 54
3.2.2 Dot blot analysis for Rab 5 in RF 55
3.2.3 Biochemical analysis of magnetically purified fraction (RF) 55
3.2.4 Determination of intravesicular pH in native and ATP treated endosomes 57
3.2.5 Electron microscopy 58
3.2.6 Protein analysis of RF by SDS PAGE 58
3.2.7 Analysis of proteolytic activity in endosome fractions 59
3.2.8 Immuno identification of endosomal proteins by Western blot analysis 60
3.2.9 Immunofluorescence confocal microscopy 65
3.2.10 Analysis of endosomal lipids 65

CHAPTER 4. DISCUSSION 68

BIBLIOGRAPHY 90