ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

VETERINARIA 64

Serum Amyloid A (SAA) as a Marker of Inflammation in the Horse

Biochemical, experimental and clinical studies

Cecilia Hultén

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

Serum amyloid A (SAA) as a marker of inflammation in the horse. Biochemical, experimental and clinical studies.

Cecilia Hultén

Akademisk avhandling som för vinnande av veterinärmedicine doktorsexamen kommer att offentligen försvaras i Ettans föreläsningssal, Klinikcentrum, SLU, Uppsala, fredagen den 3 december 1999, kl. 9.15.

Abstract

Objective assessment of infectious and non-infectious inflammation is of value in many areas of equine clinical practice and research. The aims of this thesis were to establish methods for purification, characterisation and quantitative measurement of the acute phase protein serum amyloid A (SAA) and to evaluate its usefulness as a marker of inflammation in the horse.

Equine SAA was purified using a four-step chromatographic procedure based on hydrophobic interaction chromatography, gel filtration and strong cation exchange chromatography. Further characterisation using two-dimensional electrophoresis, Western blotting and amino acid sequence analysis revealed three acute phase isoforms of equine SAA with isoelectric points of 8.0, 9.0 and 9.7 and differences in their amino acid sequences at positions 16, 44 and 59.

Purified SAA was used as primary standard in a non-competitive chemiluminescence enzyme immunoassay, in which affinity purified anti-equine amyloid A was used for coating and detection. An acute phase serum, calibrated against the primary standard, was used as working standard. The detection limit was 0.5 mg/L, and the assay had a working range of 3-1210 mg/L and could be performed in approximately three hours.

In the clinical evaluation the SAA responses in non-infectious inflammation (surgery and experimental non-infectious arthritis) and infections (acute equine influensavirus infection in adult horses and septicaemia, *Rhodococcus equi*-pneumonia and rotavirus diarrhoea in the foal) were investigated.

The evaluation revealed prominent SAA responses in non-infectious inflammation, with maximal concentrations two days after induction and a return to normal concentrations within one (castration) or two (arthritis) weeks.

Acute influensavirus infection elicited an SAA response with increasing concentrations during the first 48 hours of clinical signs of disease and a return to normal concentrations within 2-3 weeks.

Foals with bacterial infections had significantly higher SAA concentrations compared to foals with non-bacterial or uncertain diagnoses.

In conclusion, SAA responded prominently to tissue damage and infection and has the potential of being useful as a marker of inflammation in the horse.

Key words: Acute phase protein, serum amyloid A, horse, purification, two-dimensional electrophoresis, isoforms, immunoassay, surgery, arthritis, equine influensavirus, septicaemia, *Rhodococcus equi*-pneumonia, rotavirus infection.

Distribution:
Swedish University of Agricultural Sciences
Department of Clinical Chemistry
S-750 07 UPPSALA, Sweden

Uppsala 1999
ISSN 1401-6257
ISBN 91-576-5449-2

Serum Amyloid A (SAA) as a Marker of Inflammation in the Horse

Biochemical, experimental and clinical studies

Cecilia Hultén
Department of Clinical Chemistry
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 1999

Acta Universitatis Agriculturae Sueciae
Veterinaria 64

ISSN 1401-6257
ISBN 91-576-5449-2

Tryck: SLU Service/Repro, Uppsala 1999

Carpe diem!

Till minne av
min Mor, Anita

Abstract

Hultén, C. 1999. *Serum amyloid A (SAA) as a marker of inflammation in the horse. Biochemical, experimental and clinical studies.* Doctor's dissertation.
ISSN 1401-6257, ISBN 91-576-5449-2

Objective assessment of infectious and non-infectious inflammation is of value in many areas of equine clinical practice and research. The aims of this thesis were to establish methods for purification, characterisation and quantitative measurement of the acute phase protein serum amyloid A (SAA) and to evaluate its usefulness as a marker of inflammation in the horse.

Equine SAA was purified using a four-step chromatographic procedure based on hydrophobic interaction chromatography, gel filtration and strong cation exchange chromatography. Further characterisation using two-dimensional electrophoresis, Western blotting and amino acid sequence analysis revealed three acute phase isoforms of equine SAA with isoelectric points of 8.0, 9.0 and 9.7 and differences in their amino acid sequences at positions 16, 44 and 59.

Purified SAA was used as primary standard in a non-competitive chemiluminescence enzyme immunoassay, in which affinity purified anti-equine amyloid A was used for coating and detection. An acute phase serum, calibrated against the primary standard, was used as working standard. The detection limit was 0.5 mg/L, and the assay had a working range of 3-1210 mg/L and could be performed in approximately three hours.

In the clinical evaluation the SAA responses in non-infectious inflammation (surgery and experimental non-infectious arthritis) and infections (acute equine influensavirus infection in adult horses and septicaemia, *Rhodococcus equi*-pneumonia and rotavirus diarrhoea in the foal) were investigated.

The evaluation revealed prominent SAA responses in non-infectious inflammation, with maximal concentrations two days after induction and a return to normal concentrations within one (castration) or two (arthritis) weeks.

Acute influensavirus infection elicited an SAA response with increasing concentrations during the first 48 hours of clinical signs of disease and a return to normal concentrations within 2-3 weeks.

Foals with bacterial infections had significantly higher SAA concentrations compared to foals with non-bacterial or uncertain diagnoses.

In conclusion, SAA responded prominently to tissue damage and infection and has the potential of being useful as a marker of inflammation in the horse.

Key words: Acute phase protein, serum amyloid A, horse, purification, two-dimensional electrophoresis, isoforms, immunoassay, surgery, arthritis, equine influensavirus, septicaemia, *Rhodococcus equi*-pneumonia, rotavirus infection.

Author's address: Cecilia Hultén, Department of Clinical Chemistry, SLU, P. O. Box 7038, S-750 07 Uppsala, Sweden

Contents

Abbreviations,

Introduction, 11
The acute phase response, 11
The acute phase proteins, 11
Serum amyloid A (SAA), 12
Regulation of SAA synthesis, 13
Structure and characteristics of the protein SAA, 14
Function of SAA, 15
Assays for measurement of SAA, 15
Other acute phase proteins, 17
C-reactive protein, 17
Haptoglobin, 17
Fibrinogen, 18
Additional acute phase proteins in the horse, 18
Acute phase protein responses in disease, 18
SAA measurements in human medicine, 19
SAA measurements in veterinary medicine, 19
Cattle, 19
Horse, 20
Other species, 20

The basis and aims of the study, 21

Methodology, 22
Animals, sampling procedures and clinical recordings, 22
Purification and characterisation of SAA (paper I), 22
Immunisation: Animals and procedures (papers I-II), 22
Clinical evaluation of SAA as a marker of inflamation in the horse (papers II-V), 22
Reference values (paper II), 22
Surgical trauma (paper II), 22
Experimental non-infectious arthritis (papers II-III), 23
Viral infection (paper IV), 23
Infections in foals (paper V), 23
Laboratory methods, 24
Purification and characterisation of SAA (paper I), 24
Development of an immunoassay for SAA (paper II), 24
Biochemical analyses (papers III and V), 25

Haptoglobin (paper III), 25
Fibrinogen (paper III and V), 25
Total protein (paper III), 25
Serum protein electrophoresis (paper III), 25
Haematological analyses (paper V), 25
Statistical methods (papers II-V), 25

Results and discussion, 27
Purification and characterisation of equine SAA (paper I), 27
Characteristics of the immunoassay for measurement of equine SAA (paper II), 29
Clinical evaluation of SAA measurements in the horse (papers II-V), 31
SAA concentrations in healthy horses (paper II), 31
Non-infectious inflammation (papers II-III), 31
Infectious disease, 33
Viral infection (paper IV), 33
Infections in foals (paper V), 34

Conclusions, 37
Is SAA a useful marker of inflammation in the horse?, 38

References, 40

Acknowledgements, 53

Appendix

Papers I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Hultén C, Sletten K, Foyn Bruun C & Marhaug G, 1997. The acute phase protein serum amyloid A (SAA) in the horse: isolation and characterization of three isoforms. *Vet Immunol Immunopathol* 57: 215-227.

II. Hultén C, Tulamo R-M, Suominen MM, Burvall K, Marhaug G & Forsberg M, 1999. A non-competitive chemiluminescence enzyme immunoassay for the equine acute phase protein serum amyloid A (SAA) - a clinically useful inflammatory marker in the horse. *Vet Immunol Immunopathol* 68: 267-281.

III. Hultén C, Grönlund U, Hirvonen J, Tulamo R-M, Marhaug G & Forsberg M. The acute phase response during experimental non-infectious arthritis in the horse: dynamics of the inflammatory markers serum amyloid A (SAA), haptoglobin, fibrinogen and α_2-globulins. Submitted for publication.

IV. Hultén C, Sandgren B, Skiöldebrand E, Klingeborn B, Marhaug G & Forsberg M, 1999. The acute phase protein serum amyloid A (SAA) as an inflammatory marker in equine influenza virus infection. *Acta Vet Scand* (accepted for publication).

V. Hultén C & Demmers S. Serum amyloid A (SAA) as an aid in the management of infectious disease in the foal: comparison with total leukocyte count, neutrophil count and fibrinogen concentration. Submitted for publication.

Abbreviations

AA	amyloid A
ApoA-I	apolipoprotein A-I
ApoA-II	apolipoprotein A-II
APP	acute phase protein
APR	acute phase response
BNPS-skatole	3-bromo-3-methyl-2-(2-nitrophenyl-mercapto)-3H-indole
CNBr	cyanogen bromide
CRP	C-reactive protein
2D	two-dimensional
EDTA	ethylenediaminetetraacetic acid
EHV-1	equine herpes virus-1
EIA	enzyme immunoassay
ESR	erythrocyte sedimentation rate
HDL	high-density lipoprotein
HIC	hydrophobic interaction chromatography
HRP	horse radish peroxidase
IL-1	interleukin-1
IL-6	interleukin-6
kDa	kiloDalton
LPS	lipopolysaccharide
mRNA	messenger RNA
pi	post-induction
PVDF	polyvinylidene difluoride
RIA	radio-immunoassay
RID	radial immunodiffusion
RNA	ribonucleic acid
SAA	serum amyloid A
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SPE	serum protein electrophoresis
TNF-α	tumour necrosis factor-α
WHO	World Health Organization

Introduction

A substantial part of equine clinical practice and research, as well as of veterinary medicine in general, is concerned with the management of infectious and inflammatory disease. The detection, control and treatment of inflammatory processes are all areas where objective assessment of inflammation is of value. Clinical, haematological and biochemical markers of inflammation, have been, and are, used in the management of inflammation. Common to these markers is their unspecific nature in that they do not give information on the aetiology of the inflammation. This thesis deals with one of the biochemical markers of inflammation, the acute phase protein serum amyloid A (SAA) in the horse. In the following, a background and a context for this work are given.

The acute phase response

Inflammation is the response of the body to insults leading to tissue damage. It is designed to repair the damage and limit its spread (Gallin et al., 1992). This local process leads, by way of mediators (cytokines), produced by inflammatory and other cells at the site of inflammation, to a systemic response including an array of changes that produce an alteration of homeostasis. This systemic response is considered a part of the natural immune system and is referred to as the acute phase response (APR) (Kushner & Mackiewicz, 1993). It comprises behavioural, clinical, haematological, metabolic and biochemical changes intended to quickly adapt the body to a defence situation and make it better suited to overcome the insult. In spite of its designation as an acute phenomenon, the response is maintained as long as the inflammatory process is active. Therefore, it may be persistent in chronic disease (Gabay & Kushner, 1999). A persistent APR may be harmful to the body since alterations of homeostasis which are appropriate from a defence perspective can have unwanted secondary effects on the body if very pronounced and/or maintained over an extended period of time. The regulation of the APR therefore has to be tight and it is known that several repressive and inhibitory systems work in parallel to control the response (Jensen & Whitehead, 1998). However, the detailed mechanisms of these systems are not yet entirely delineated.

The acute phase proteins

One prominent feature of the APR is the rearrangement of protein synthesis in the liver following stimulation of the hepatocytes by cytokines. Some proteins, the positive acute phase proteins, increase in concentration at the expense of others, the negative acute phase proteins. The definition of an acute phase protein (APP) is a protein which increases (positive APP) or decreases (negative APP) by at least 25% during inflammation. The negative APPs will not be further discussed in this work and the term APP will be used for positive APPs. The degree of concentration increase differs considerably between the APPs and for

this reason, they are sometimes grouped as minor, moderate or major APPs according to their relative concentration increase following inflammatory stimuli (Kushner, 1982; Van Leeuwen & Van Rijswijk, 1994). There is also a species variation in the degree of concentration increase of specific proteins as well as in whether a protein is expressed as an APP or not (Eckersall, 1992).
Several cytokines have been shown to stimulate APP production *in vitro* or *in vivo*, but interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) are considered the main inducers of the APP synthesis. In addition to cytokines, hormones and growth factors also influence the APP production. Glucocorticoids have an important regulatory function, by enhancing APP synthesis and inhibiting the production of cytokines (Koj et al., 1993) .
The APPs constitute a heterogeneous group of proteins with different, and in some cases unknown, functions and expression patterns. In general, their functions are considered to be beneficial to the inflammatory process, either by being mainly pro-inflammatory or anti-inflammatory (Gabay & Kushner, 1999).
The expression and production of APPs are, alike the APR in general, stimulated unspecifically and regardless of type of inflammatory stimulus. Bacterial, viral and parasitic infections as well as non-infectious inflammation and trauma may elicit the production of APPs. However, bacterial infections and sepsis are considered to give the most prominent responses (Whicher et al., 1993, Gruys et al., 1994). However, it seems that the disease activity and the extent of the inflammatory process might be equally, or more, important for the plasma concentration of an APP as the type of stimulus (Whicher et al., 1993).

Serum amyloid A

Serum amyloid A (SAA) was first discovered in the 1970s when antiserum to the protein amyloid A (AA) from amyloid deposits of reactive amyloidosis, was found to cross react with serum from patients with inflammatory diseases (Levin et al., 1973; Husby & Natvig, 1974). Reactive or secondary amyloidosis is a potentially fatal disease during which AA is deposited in different tissues of the body, predominantly the liver and the kidneys, in the course of chronic inflammatory processes (Husby, 1994; Kisilevsky & Young, 1994). The serum protein identified was called " serum amyloid A" and it was later established that SAA actually is the precursor of AA, the protein in the amyloid deposits of reactive amyloidosis (Husebekk et al., 1985). Further interest was directed towards SAA as its potential as a marker of inflammation became evident (McAdam et al., 1975; Rosenthal & Franklin, 1975).
SAA is one of the major APPs in that its serum concentration can increase up to several thousandfold in response to inflammatory stimuli. This has been established based on investigations of mRNA expression or serum concentration during inflammation in man (Marhaug et al., 1983; Malle & De Beer, 1996) as well as in several other species, including mouse (Lowell et al., 1986; De Beer et al., 1992), rabbit (Liepnieks et al., 1991; Rygg et al., 1993a), mink (Marhaug et al., 1990; Rygg et al., 1993b), dog (Sellar et al., 1991; Yamamoto et al., 1994),

cattle (Husebekk et al., 1988; Horadagoda et al., 1993), cat (Kajikawa et al., 1999), sheep (Syversen et al., 1994a) and horse (Husebekk et al., 1986; Pepys et al., 1989). The rat holds a unique position in this respect by lacking circulating SAA although expressing an SAA-like mRNA in the liver and several other organs during inflammation (Baltz et al., 1987; Meek & Benditt, 1989). In the pig, a 10-13 kDa-protein reacting with antiserum to human SAA has been detected during inflammation although this protein has not been further characterised (Heegaard et al., 1998). SAA has been identified as an APP also in non-mammals such as salmonids (Jensen et al., 1997) and ducks (Ericsson et al., 1987; Guo et al., 1996).

Regulation of SAA synthesis

The term "serum amyloid A" designates a group of proteins that in almost all species investigated are the products of several genes. The SAA gene family has been studied in detail at the genomic level in mouse (Yamamoto et al., 1985; Lowell et al., 1986; Stearman et al., 1986; Butler & Whitehead, 1997) and man (Sipe et al., 1985; Kluve-Beckerman et al., 1988; Sack & Talbot, 1989; Betts et al., 1991; Steel et al., 1993), and the SAA genes of rabbit (Tatum et al., 1990; Ray & Ray, 1991; Rygg et al., 1991), Syrian hamster (Webb et al., 1989; Gervais & Suh, 1990), mink (Marhaug et al., 1990), rat (Meek & Benditt, 1989), salmonids (Jensen et al., 1997) and duck (Guo et al., 1996) have also been investigated. Most of the protein products of these genes are APPs, but in humans a constitutive SAA gene has been detected that does not respond to acute phase stimuli (Whitehead et al., 1992; Steel et al., 1993). The acute phase SAA genes are mainly expressed in hepatocytes but in man (Urieli-Shoval et al., 1994; Yamada et al., 1996; Urieli-Shoval et al., 1998; Kumon et al., 1999), mouse (Meek & Benditt, 1986; Meek et al., 1989; Marhaug et al., 1997), rabbit (Rygg et al., 1993a; Marhaug et al., 1997), mink (Marhaug et al., 1990; 1997), goat and sheep (Sack & Zink, 1992) extrahepatic expression of SAA has been described. Differential expression patterns for the acute phase SAA genes concerning the site and degree of expression have been reported *in vivo* during inflammation in mouse (Meek & Benditt, 1986; Rokita et al., 1987;) and rabbit (Rygg et al., 1993a; Ray & Ray, 1997). The basis for this variation in gene expression is not well understood thus far. Studies into the regulatory mechanisms of SAA and other APPs indicate that these differences can come about due to regulatory elements and feedback loops which influence the genes individually (Jensen & Whitehead, 1998).

The main stimulators of SAA gene expression are, as for the acute phase proteins in general, the cytokines IL-1, IL-6 and TNF-α. A large number of studies have been done *in vivo* and *in vitro* to establish the mutual relationship between these cytokines when it comes to stimulation of the SAA expression. These studies seem to verify that IL-6 is a potent inducer of SAA. However, IL-6 cannot work on its own but needs IL-1 in order to function. Under the influence of IL-1, which

also stimulates the SAA expression by itself, IL-6 works synergistically to enhance the expression of SAA (Marhaug & Dowton, 1994).
To date there have been no investigations into equine SAA on the genomic level.

Structure and characteristics of the SAA protein

Acute phase SAA is an apolipoprotein, which means that it is transported in plasma attached to lipids in a lipoprotein complex (Benditt & Eriksen, 1977; Benditt et al., 1979). SAA is mainly complexed to the third fraction of high-density lipoprotein (HDL_3) but can be found in all lipoprotein fractions (Marhaug et al., 1982; Eriksen & Benditt, 1984). During the APR, when increasing amounts of SAA appear in the circulation, apolipoprotein A-I and apolipoprotein A-II in the HDL-particle II are displaced by SAA, which may account for most of the protein content of the HDL-particle during an APR (Clifton et al., 1985).
Equine SAA is a protein of approximately 11 kDa with 110 amino acid residues (Husebekk et al., 1986; Sletten et al., 1989). The circulating protein exhibits three heterogeneous positions in its amino acid sequence rendering the existence of multiple circulating isoforms possible (Sletten et al., 1989). The partial or complete amino acid sequences of acute phase SAAs have been established in man (Sletten et al., 1983; Dwulet et al., 1988; Beach et al., 1992), mouse (Hoffman et al., 1984; De Beer et al., 1991; De Beer et al., 1992), cattle (Rossevatn et al., 1992; Alsemgeest et al., 1995a), rabbit (Liepnieks et al., 1991; Syversen et al., 1993a), mink (Syversen et al., 1993b), sheep (Syversen et al., 1994a) and duck (Ericsson et al., 1987). In all these species, except the rabbit and the duck, heterogeneous positions in the amino acid sequence have been noted and in some cases the actual circulating isoforms have been separated. These proteins show great similarities between species and a specific region of 20 amino acid residues is very well conserved with almost identical primary structure in all species investigated. This high degree of homology and preservation suggests that the conserved region in particular, but also SAA in general, has an important function (see below).
The reason for the interest in this field among SAA researchers is at least twofold. First, there is a species difference as to whether or not the deposition of amyloid during reactive amyloidosis is isoform-dependent (Marhaug & Dowton, 1994). Understanding these mechanisms is of great importance for management of amyloidosis. Second, and more within the scope of this thesis is the finding that these isoforms might be differentially synthesised following different acute phase stimuli. Although most of the studies regarding differential regulation have been done on the genomic level studying expression of the genes, there have been some investigations into the dynamics of the circulating isoforms as well. These studies have given less consistent results compared with the studies of gene expression, but in humans different dynamics over time for circulating isoforms of SAA have been shown (Raynes & McAdam, 1991; Kluve-Beckerman et al., 1997). On the other hand, an investigation of the SAA isoform distribution in mice following different acute phase stimuli indicated similar

production of the circulating isoforms regardless of stimulus (Foyn Bruun et al., 1998).

Function of SAA

Increasing attention is beeing given to the question of the function of SAA. The opinion that circulating acute phase SAA play an important role in hte inflammatory process has been advocated for a long time and is based on the fact that the concentration of SAA increases rapidly and prominently upon stimulation; that the SAA genes have been well preserved through evolution; that there are regions of the amino acid sequence which are identical, or almost identical, in all species investigated and finally; that no human subject lacking SAA has been described (Jensen & Whitehead, 1998). Possibly opposing this opinion is the fact that in the rat, no SAA has been found in the circulation although the SAA genes are present and have substantial homology with those in other species. Several functions have been ascribed to acute phase SAA, most of which are related to the inflammatory process, but so far, no conclusive picture of SAA function has evolved.

The reports on proposed action of SAA in the inflammatory process might seem somewhat contradictory. Some investigators have found immunosuppressive effects of SAA on neutrophil function and cell adhesion (Linke et al., 1991; Preciado-Patt et al., 1994; Gatt et al., 1998) whereas others have shown that SAA promotes the local inflammation by functioning as a chemo-attractant (Badolato et al., 1994; Olsson et al., 1999). The experiments leading to these conclusions have been done with different forms of SAA (recombinant, purified or SAA-HDL) or synthetic peptide fragments identical to sequences in the SAA molecule and at different SAA concentrations. Several authors have demonstrated the inhibitory effect of SAA on different types of platelet aggregation (Zimlichman et al., 1990; Levartowsky & Pras, 1991; Syversen et al., 1994b). The possible role of SAA as a modulator of cholesterol metabolism and transport in inflammatory and atherosclerotic lesions has gained much interest and several reports on different aspects of this subject have been published (Kisilevsky, 1991; Kisilevsky & Subrahmanyan, 1992; Meek et al., 1994; Shainkin-Kestenbaum, 1996). The basis for these investigations is the fact that fat emanating from the destroyed tissue accumulates in the atherosclerotic lesions and thereby increases the need for lipid transport from the inflammatory site . Lately th ability of SAA to induce synthesis of IL-1β, IL-1 receptor antagonist and soluble TNF receptor-II was reported and interpreted as indicating an immunoregulatory function (Patel et al., 1998)

Assays for measurement of SAA

Due to the association of native SAA with HDL and the different characteristics of this SAA-HDL compared with purified SAA, developing and standardising assays for SAA have been a major challenge for researchers wanting to measure SAA concentrations in plasma or other body fluids. In trying to overcome the

problems of standardisation of SAA assays a number of assay systems relying on different reagents have been developed over the years. The earliest investigations measuring human SAA employed semi-quantitative immunodiffusion techniques using anti-AA antisera and purified AA to identify SAA in serum (Levin et al., 1973; Husby & Natvig, 1974). These were followed by radio-immunoassays (RIAs) using the same reagent combinations (Sipe et al., 1976; Van Rijswijk, 1981). The next step was the introduction of enzyme immunoassay (EIA) techniques and during the following years the SAA assay systems were further developed with the use of purified SAA for standardisation and the introduction of monoclonal antibodies in the assays (Marhaug, 1983). The problem with the different conformation of native and purified SAA was addressed either by changing the conformation of SAA in the samples using delipidation (Dubois & Malmendier, 1988; Saïle et al., 1988), heat (Sipe et al., 1989) or chemical treatment (McCormack et al., 1996), or by using different forms of native SAA as a standard (Godenir et al., 1985; Yamada et al., 1989; Hachem et al., 1991). Further improvements have been achieved by using sequence specific antibodies (Hachem et al., 1991; Časl & Grubb, 1993) and recombinant SAA (McCormack et al., 1996). Attempts have also been made to shorten the analysis time by automation of the EIA technique (Wilkins et al., 1994) and by developing nephelometric assays (Hocke et al., 1989; Yamada et al., 1993).

To improve the agreement in SAA determinations between different laboratories an international standard of human SAA has been produced and evaluated, and is now available as a World Health Organization (WHO) standard (Poole et al., 1998)

Since the middle of the 1980s the flow of publications on human SAA assays has been paralleled by development of SAA assays for some animal species. Most of these have been EIAs, the earliest of which were designed for SAA measurement in serum from mice (Wood et al., 1982; Zuckerman & Surprenant, 1986). The first method for measuring SAA in serum from cattle relied on affinity-purified anti-bovine amyloid A antibodies and SAA concentrations were reported in relative units (Boosman et al., 1989). Horadagoda and co-authors (1993) developed a quantitative assay using an anti-human SAA and purified bovine SAA. Feline SAA has been measured utilising a feline amyloid A/anti-feline amyloid A system in radial immunodiffusion (DiBartola et al., 1989) and by EIA using an anti-canine SAA antibody and feline SAA (Kajikawa et al., 1996). Yamamoto et al. (1994) measured canine SAA by an EIA technique with species-specific antibodies. In the horse, SAA has been measured exclusively with species-specific reagents using electroimmunoassay (Pepys et al., 1989), radial immunodiffusion (RID) (Nunokawa et al., 1993) and EIA (Satoh et al., 1995).

The need for interlaboratory harmonisation of SAA and other APP assays in veterinary medicine has been discussed and measures have been taken to try to establish a similar network for SAA assay calibration between veterinary laboratories as in human medicine (Eckersall et al., 1999)

Other acute phase proteins

C-reactive protein

C-reactive protein (CRP) is the prototype APP and it has been extensively studied over the years. A short review of this protein is therefore given here although it has not been measured in the investigations presented in this thesis. C-reactive protein derives its name from its ability to precipitate pneumococci by binding to the C-polysaccharide in the cell walls of the bacteria (Tillett & Francis, 1930). Since this discovery was made, other important functions have been ascribed to CRP. It acts as an opsonin for bacteria, parasites and immune complexes and it activates the classical pathway of the complement cascade (Agrawal et al., 1993). In addition it has the ability to bind chromatin and this function is considered important in preventing nuclear-antigen autoimmunity in connection with release of nuclear material from damaged cells (Robey et al, 1984). C-reactive protein belongs to the pentraxins (Osmand et al., 1977), a group of proteins that have been very well preserved through evolution. CRP-like pentraxins have been found in all mammals tested, in birds, in fish and in the horseshoe crab which is an ancient species still living on earth (Baltz et al., 1982; Robey & Liu, 1981). As for SAA this has led to the conclusion that CRP is important for the defence of the organism.

CRP is the classical major APP in human medicine and has been used extensively in clinical practice. In the horse, however, CRP only reacts moderately during inflammation (Yamashita et al., 1991). Dog (Dillman & Coles, 1966; Caspi et al., 1987), rabbit (Giclas et al., 1985) and pig (Heegaard et al., 1998) show increases in CRP comparable to those seen in humans, whereas CRP in the mouse only is a minor APP (Whitehead et al., 1990). There are conflicting reports on whether CRP in cattle is an APP or not (Maudsley et al., 1987; Morimatsu et al., 1991; Schrodl et al., 1995).

Haptoglobin

Haptoglobin is a glycoprotein showing α_2-mobility in serum protein electrophoresis. It is present in normal serum in concentrations of up to 2 g/L in most species investigated, including man (Roy et al., 1969; Elson, 1974), horse (Harvey, 1976; Willett & Blackmore, 1979), dog (Harvey, 1976), cat (Harvey, 1976) and pig (Eckersall et al., 1996; Lipperheide et al., 1998). It thus differs from the major APPs SAA and CRP by being present in significant concentrations also in healthy subjects. During inflammatory processes the serum haptoglobin concentration increases moderately in these species. However, in cattle haptoglobin is a major APP present in undetectable or very low concentrations in healthy animals and showing large relative increases during inflammation (Skinner et al., 1991; Hirvonen et al., 1999).

Haptoglobin binds haemoglobin strongly and the complex is rapidly removed from circulation by the reticuloendothelial system (Hwang & Greer, 1980). The subsequent decreased concentrations in serum have been used in the assessment of haemolytic disease (Nosslin & Nyman, 1958; Wilke et al., 1992). In addition

to binding haemoglobin, a number of regulatory functions related to the immune system have been reported for haptoglobin (Dobryszycka, 1993).
The most common way of estimating haptoglobin in serum has been to measure the haemoglobin-binding capacity. In the horse electrophoresis (McGuire & Henson, 1969), gel filtration (Allen & Archer, 1971) spectrophotometry (Harvey, 1976) and peroxidase activity (Willett & Blackmore, 1979) have been used to quantitate the haemoglobin-haptoglobin complex. The advantage of methods based on haemoglobin-binding capacity is that they are not species specific, but can be used for several species with slight adjustment. More recently, an immunoassay for equine haptoglobin has been developed (Kent & Goodall, 1991)

Fibrinogen

Fibrinogen, factor I of the coagulation system, is the circulating precursor of fibrin. The insoluble fibrin is the end product of the coagulation cascade and polymerises at sites of vascular damage to constitute a mesh to which cells and other components necessary for wound healing can adhere (Fuller, 1993). Due to the important function of fibrin in wound healing, it is logical that its precursor is an APP (Andreotti et al., 1999). In addition to the horse (Andrews et al., 1994), increased concentrations of fibrinogen during inflammatory processes have been reported in most species commonly encountered in veterinary medicine including cattle (Conner et al., 1988), sheep (Pfeffer & Rogers, 1989), pig (Odink et al., 1990), dog (Ganroth, 1973; Millis et al., 1992) and cat (Gouffaux et al., 1975). In all these species fibrinogen can be considered as a moderate APP with normal concentrations in the order of up to 4-7 g/L in healthy animals.

Additional acute phase proteins investigated in the horse

In addition to the APPs described above, α_1-acid glycoprotein (orosomucoid) (Taira et al., 1992), α_2-macroglobulin (Pellegrini et al., 1983; Milne et al., 1991) and ceruloplasmin (Smith & Cipriano, 1987; Milne et al., 1991) have been isolated from equine serum and identified as minor APPs.

Acute phase protein responses in disease

One of the foundations for the use of APPs as markers of inflammatory disease was laid as early as 1914 when Von den Velden noticed increased concentrations of fibrinogen in response to experimental inflammation. This finding was further investigated by Fåhraeus (1921), and his observations led to the development of erythrocyte sedimentation rate (ESR) as a marker of inflammatory disease. Todat the ESR is still widely used as a diagnostic tool in human medicine.
The APPs are used to (Whicher et al., 1993; Van Leeuwen & Van Rijswijk, 1994):

- detect infectious or inflammatory conditions and distinguish them from non-inflammatory processes,

- distinguish bacterial from other infections or inflammatory diseases with some certainty
- monitor response to treatment and
- aid in the management of a case concerning prognosis and outcome.

The most commonly analysed specific APP in human medicine is CRP and in veterinary medicine, fibrinogen. In veterinary medicine, the future use of APP determinations as an aid in health and welfare control at the herd level (Alsemgeest et al., 1995c; Lipperheide et al., 1998) and in animal and meat inspection at slaughter have been discussed and investigated (Gruys et al., 1993; Toussaint et al., 1995; Saini et al., 1998).

SAA measurements in human medicine

Only a few years after SAA had been identified as an APP, reports were published of SAA being measured in patient sera. Gorevic and co-authors (1976) demonstrated markedly elevated SAA concentrations in patients with bacterial infections. In patients responding to treatment the SAA concentrations decreased promptly. The SAA response following surgery was less pronounced but elevated SAA concentrations were seen during 2-3 weeks post-operatively. Since then investigations have been presented, in which SAA has been suggested to be useful in discriminating between solid tumours and tumours with metastases (Rosenthal & Sullivan, 1979; Biran et al., 1986) and in monitoring rejection after transplantation of kidney (Maury et al., 1983; Müller et al., 1992), liver (Feussner et al., 1994) and heart (Müller et al., 1998). Likewise, viral infections (Shainkin-Kestenbaum et al., 1982; Miwata et al., 1993) and myocardial infarction (Marhaug et al., 1986; Časl et al., 1995) give rise to prominent increases of the SAA concentration. Due to the fact that assays for CRP are easily available and to the largely similar behaviour of CRP and SAA in many conditions (Maury, 1985; Fukuda et al., 1998), the routine use of SAA measurements in human medicine is limited, although SAA seems to be a more sensitive marker than SAA, especially in viral infections (Smith et al., 1992; Nakayama et al., 1993; Liuzzo et al., 1994; Fukuda et al., 1998).

SAA measurements in veterinary medicine

Cattle

So far, in veterinary medicine, the SAA responses in disease, both experimentally induced and naturally occurring, have been most widely investigated in cattle. Boosman and co-authors (1989) reported increased SAA concentrations after intravenous endotoxin administration to cows. Following experimentally induced *Pasteurella hemolytica* pneumonia in calves, SAA concentrations started to rise two hours after inoculation and rose prominently to maximal concentrations on day 2 after inoculation (Horadagoda et al., 1993; Horadagoda et al., 1994). Elevated SAA concentrations have been found in connection with many common, naturally occurring diseases in cattle (Van der Kolk, 1991; Gruys et al.,

1993; Alsemgeest et al., 1994), and Horadagoda and co-authors (1999) have recently demonstrated the use of SAA measurements for discriminating between acute and chronic inflammatory processes. Chronically stressful housing conditions due to inappropriate flooring gave rise to increased SAA concentrations in calves (Alsemgeest et al., 1995). Parturition gave rise to an APR in the cow indicated by high SAA concentrations. Maximal values occurred at 24 hours after parturition, whereas no significant increase in the foetus or in the newborn calf was detected (Alsemgeest et al., 1993; Alsemgeest et al., 1995b).

Horse

The investigations of SAA responses during inflammatory conditions in the horse have given grossly similar results as for cattle. Elevated concentrations of equine SAA have been reported post-operatively, with maximal concentrations 2 days after surgery (Pepys et al., 1989; Satoh et al., 1995). In experimental infection with equine herpes virus-1 (EHV-1) (Pepys et al., 1989) a prominent SAA response was noted that coincided with fever and viremia. Pepys and co-authors (1989) and Satoh and co-authors (1995) also found increased SAA concentrations in several infectious and inflammatory conditions and the use of SAA measurements as an aid in the management of neonatal foal diseases has been suggested (Chavatte et al., 1991).

Other species

In the cat, high SAA concentrations have been noted in connection with experimental non-infectious inflammation (Kajikawa et al., 1999) and in cats with various diseases other than amyloidosis (DiBartola et al., 1989; Kajikawa et al., 1999).

In dogs, *Bordetella bronchiseptica* infection has been demonstrated to give rise to increased SAA concentrations in the order of 20 times the baseline level (Yamamoto et al., 1994).

In the pig, a 10-13 kDa protein showing immunoreactivity with human anti-SAA antiserum increases in concentration following *Actinobacillus pleuropneumoniae* infection in swine (Heegaard et al., 1998).

The basis and aims of the study

The basis of the present study was the need for sensitive and objective markers for inflammatory disease in equine practice and research. Equine research dealing with performance and infectious disease, the management of the equine athlete, the intensive treatment of neonatal diseases in the foal and the health control of horses to be introduced into new environments are all possible fields of application for such markers.
The aims of the present study were therefore:

- to make equine SAA available for studies by establishing methods for its purification, characterisation and quantitative measurement;

- to monitor and characterise the SAA response to non-infectious and infectious inflammatory stimuli under experimental or natural conditions;

- to compare the SAA response after non-infectious and infectious stimuli with the responses of presently used inflammatory markers in the horse; and

- to assess the clinical usefulness of SAA as an inflammatory marker in the horse.

Methodology

Brief descriptive presentations of the animals, designs and methods used are given in the following. Detailed information and relevant references are available in the papers indicated.

Animals, sampling procedures and clinical recordings

All use of animals included in this thesis was approved by the authorities concerned in Sweden, Norway and Finland.

Purification and characterisation of SAA (paper I)

Serum was collected by jugular venipuncture from five horses with severe inflammatory processes due to bacterial infection (n=3), laminitis (n=1) and bowel surgery (n=1). These sera were used for the purification and characterisation of SAA. Each serum was investigated separately. A serum sample from a clinically healthy horse was used for comparisons.

Immunisation: Animals and procedures (paper I and II)

Rabbits were utilised for production of the antiserum used for characterisation of SAA (paper I) and for the SAA assay (paper II). The antiserum used in paper I was produced by means of weekly injections of crude amyloid fibrils from equine liver mixed with purified AA from the same source in complete Freund's adjuvant (Husebekk et al., 1986). The antiserum for the SAA assay was produced in six loose-housed rabbits which were immunised monthly with the same antigen mixture as above (paper II).

Clinical evaluation of SAA as a marker of inflammation in the horse (paper II-V)

Reference values (paper II)

To establish reference values for SAA in the horse, serum samples from clinically healthy horses (n=170) were used. The vast majority of these horses were Standardbred trotters.

Surgical trauma (paper II)

Healthy horses (n=7) undergoing closed normal castration under general anaesthesia were sampled before and after surgery and on days 1, 2, 4, 7 and 14 post-operatively. The horses were examined at each sampling occasion for local (swelling, stiffness, exudation) and systemic (fever, depression) signs of infection.

Samples were analysed for total leukocyte and differential counts, fibrinogen concentration, total protein concentration and serum protein electrophoresis (SPE) (unpublished results) in addition to SAA.

Experimental non-infectious arthritis (paper II-III)

Arthritis of the right midcarpal joint was induced in 24 Standardbred horses by injecting amphotericin B. Blood samples were taken by jugular venipuncture before and 8, 16, 24 and 36 hours post-induction (pi) and thereafter on days 2, 3, 4 , 5 and 15 pi. Clinical recordings included local and systemic signs of pain and inflammation. The samples were analysed for SAA, haptoglobin, fibrinogen and α_2-globulins.

Viral infection (paper IV)

Acute (i.e. less than 48 h of clinical signs) and convalescence (once between 11 and 22 days after the acute stage sampling) blood samples were taken from 70 horses suffering from equine influenza virus infection. The infection was verified by seroconversion and by detection of virus-infected cells in nasal secretions. Clinical signs (cough, nasal discharge and body temperature) were recorded during the trial. Samples were analysed for SAA.

Infections in foals (paper V)

Hospitalised foals which either had clinical signs indicating infectious disease or in which infectious disease could not be immediately ruled out, were blood sampled on admission to the Equine Clinic, Department of Large Animal Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden and thereafter daily or every second day during their stay. They were grouped according to their main presenting symptoms, which were neonatal weakness (n=9), pneumonia (n=6) and diarrhoea (n=10). Among the neonatally weak foals, two had positive blood cultures, and were euthanised due to lack of response to treatment and poor prognosis. Three foals had ambiguous blood culture results, and they recovered and were subsequently discharged from the clinic. Three neonatally weak foals had negative blood cultures, and of these all but one, that suffered from a ruptured urinary bladder, recovered and were discharged. One foal suffered from EHV-1 infection and had a negative blood culture. This foal died. All pneumonias were due to *Rhodococcus equi*-infection and of the six foals admitted to the clinic, two were euthanised due to lack of response to treatment, one died and three recovered. Four of the diarrhoeic foals had rotavirus infection, and all ten foals with diarrhoea recovered. Clinical recordings, diagnostic efforts and treatments followed the routines of the clinic. Samples were analysed for total leukocyte and differential counts and fibrinogen concentration in addition to SAA.

Laboratory methods

Purification and characterisation of SAA (paper I)

SAA was purified in a four-step chromatographic procedure including hydrophobic interaction chromatography (HIC), gel filtration and ion exchange chromatography. The HIC was done under dissociating conditions using a buffer containing 4 M guanidine hydrochloride. Bound material from this step was further purified using gel filtration in two steps. Eluted material chosen for further purification was separated using a strong cation exchanger and eluted under a linear salt gradient. Purity and molecular mass were monitored during the purification procedure using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

To further characterise the purified protein and to obtain material for amino acid sequence analysis, two-dimensional (2D) electrophoresis followed by semi-dry blotting to polyvinylidene difluoride (PVDF)-membranes and nitro cellulose membranes was performed. Sequencing of purified protein was done by way of Edman degradation, where necessary combined with cyanogen bromide (CNBr) or 3-bromo-3-methyl-2-(2-nitrophenyl-mercapto)-3H-indole (BNPS-skatole) cleavage.

Isoelectric points were assessed from the position of the protein spots on the 2D gels.

Development of an immunoassay for SAA (paper II)

Amino acid composition in solutions of purified SAA was determined and used to verify the purity of the protein and to determine the protein concentration of the sample. These solutions of purified SAA were used as primary standard for calibration of the assay.

Antiserum was produced as described above and was affinity purified using AA as ligand. Specificity for equine SAA was tested using Western blotting of 2D gels with separated, partly purified acute phase serum from the first (HIC) purification step. The affinity-purified antibody was conjugated to horse radish peroxidase (HRP).

The SAA assay was designed as a non-competitive (immunometric) chemiluminescence sandwich EIA in which the affinity purified antibody was used both for coating of the microtiter plates and for detection. In the latter case it was conjugated to HRP. A pooled acute phase serum was calibrated against the primary standard and was used as working standard in the assay. All standards, controls and samples were assayed in duplicate. Controls and samples were uniformly diluted 1:50. The assay was performed using the Amerlite System (Ortho Clinical Diagnostics, Amersham, UK).

Biochemical analyses (papers III and V)

Haptoglobin (paper III)

Haptoglobin was measured as haemoglobin-binding capacity of serum using a method based on detection of peroxidase activity (Owen, 196O; Jones & Mould, 1984).

Fibrinogen (paper III and V)

The plasma fibrinogen concentration was determined in etylenediaminetetraacetic acid (EDTA)-plasma using a kinetic method (Boehringer Mannheim Fibrinogen, Mannheim, Germany) (Becker et al., 1984) which measures increasing turbidity of the sample following activation of the clotting with a snake venom (batrobaxin). The assay was run on an automated analyser (Coba´s Mira, Hoffmann-La Roche Ltd, Basel, Switzerland).

Total protein (paper III)

Total protein concentration in serum was determined using the biuret method (Roche Unimate 7, Roche Diagnostic Systems, Switzerland) on an automated analyser (Coba´s Mira, Hoffmann-La Roche Ltd, Basel, Switzerland).

Serum protein electrophoresis (papers III)

Serum protein electrophoresis was run on an agarose gel system using barbiturate buffer at pH 8.6 (Paragon SPE System, Beckman Instruments Ltd, Fullerton, USA). The protein pattern was divided into six fractions; albumin, α1-globulins, α2-globulins, β1-globulins, β2-globulins and λ-globulins according to standard procedures for SPE. The relative protein content of each electrophoretic fraction was multiplied by the total serum protein concentration of the sample to obtain absolute concentrations for each fraction.

Haematological analyses (paper V)

Leukocytes were counted using an automated cell counter (Sysmex F-800, Toa, Kobe, Japan). Differential counts were done manually counting 100 cells on May-Grünewald Giemsa stained blood smears. The leukocytes were classified as band neutrophils, segmented neutrophils, eosinophils, basophils, lymphocytes and monocytes.

Statistical methods (papers II-V)

The upper reference limit for SAA was calculated as the value corresponding to the 97.5th percentile of the sample (paper II).

The SAA concentrations of the horses suffering from equine influenza infection were evaluated using the general linear models procedure of the SAS software (SAS Version 6.12, SAS Institute Inc., Cary, NC, USA). A model was designed

in which the factors having significant influence on the results were incorporated. Comparisons between acute and convalescent SAA concentrations and between infected and normal horses were done non-parametrically using Wilcoxon matched pairs signed rank sum test and the Mann-Whitney test, respectively (paper IV).

One-way analysis of variance, followed by Bonferroni correction for multiple comparisons was used to evaluate the dynamics of SAA, haptoglobin, fibrinogen and α_2-globulins after arthritis induction (paper III). The relationship between the markers was assessed by calculating Spearman's correlation coefficients.

Comparisons between groups of foals with different clinical signs were made non-parametrically using the Mann-Whitney test (paper V).

Results and discussion

In the following the main results of each investigation is presented and discussed. For the detailed results, the reader is referred to the respective papers.

Purification and characterisation of equine SAA (paper I)

Hydrophobic interaction chromatography (HIC) was used as the initial purification step for SAA from equine acute phase serum in this investigation. When separated by gel filtration, the protein material eluted from the initial HIC column contained a peak with an approximate molecular mass of 14 kDa, which was not present in serum from a healthy horse. Further purification of the protein material in this peak by strong cation exchange chromatography under a linear salt gradient yielded several small peaks representing impurities followed by one major peak eluted at the end of the gradient. The protein in this major peak was identified as equine SAA using NH_2-terminal amino acid sequence analysis.

The major peak from the ion exchange chromatography was further investigated using 2D electrophoresis and was shown to contain three acute phase isoforms of equine SAA. They were identified by their approximate isoelectric points as equine SAA pI 8.0 (eSAApI 8.0), eSAApI 9.0 and eSAApI 9.7. Partial amino acid sequence analysis revealed differences in the amino acid sequence between the isoforms at positions 16, 44 and 59. These are the positions in which Sletten and co-authors (1989) found heterogeneities when they determined the primary structure of equine SAA.

The purification method used in this work is a modification of the method of Raynes & McAdam (1988). The HIC gel works by binding to hydrophobic areas on the protein surface and bound material is eluted by reducing the strength of the hydrophobic bonds using reduced ionic strength or reduced polarity (Eriksson, 1989). The most hydrophobic area of the SAA molecule is constituted by the first 11 amino acids at the NH_2-terminal end of the sequence. Although not identical, this region shows great homology between species (Turnell et al., 1986). SAA is eluted from the column free from lipids and thus the HIC step provides partial purification from other proteins and delipidation in the same procedure. The subsequent purification steps are aimed at removing proteins of differing molecular mass (gel filtration) and, finally, proteins with a similar molecular mass as SAA, but with other isoelectric points (ion exchange).

The methods that have been used to purify SAA all include a step where the SAA molecule is dissociated from the lipoprotein complex. Initially, human SAA was purified using repeated gel filtration in formic acid after precipitation with an anti-AA antiserum (Anders et al., 1975). This method has also been employed in the purification of equine SAA although the initial precipitation step was omitted by these authors (Nunokawa et al., 1993). However, as can be seen in the ion exchange chromatogram presented in our paper, SAA purified by HIC followed by repeated gel filtration still contains proteins other than SAA (peaks I-VI). This

was first established by Marhaug & Husby (1981) who found prealbumin and albumin in the preparation from gel filtration.
Some research groups, interested in the association of SAA to HDL developed a purification method based on ultracentrifugation in salt gradients (Skogen et al., 1979; Eriksen & Benditt, 1980). This method was used for the first studies of SAA in the horse (Husebekk et al., 1986) and produced pure SAA, but had the disadvantages of being laborious and time-consuming. Consequently, for applications where isolation of HDL was not necessary the HIC-based method for purification of SAA was developed. For the purification of equine SAA we modified this method by running the HIC step without a gradient and by using a strong cation exchanger in the last purification step. Using a cation exchanger proved necessary due to the high isoelectric points of equine SAA.
Theoretically, there are eight possible combinations of the amino acids present in the heterogeneous positions of equine SAA. Three acute phase SAA isoforms were identified in our investigation. The possible existence of constitutive SAA isoforms present in low concentrations and demonstrating other properties than the isoforms described in our work cannot, however, be ruled out. Co-migration of isoforms in the same protein spots during 2D electrophoresis has been described previously (Foyn Bruun et al., 1994; Foyn Bruun et al., 1995), but was not found in our study, where each isoform was clearly separated from the others. The fact that eSAApI 8.0 and eSAApI 9.0 were separated although they have the same theoretical isoelectric point (pI 8.5, see paper I) might be explained by posttranslational differences between the proteins (Righetti, 1983; Glitz, 1992).
Two-dimensional electrophoresis provides a means of studying isoforms of SAA without the need for immunological techniques in species where antisera against SAA isoforms are not available. It has been used to study acute phase SAA isoforms in several species with (Foyn Bruun et al., 1993; Foyn Bruun et al., 1995), or without (Bini et al., 1996; Doherty et al., 1998) HIC as an initial purification step before electrophoresis.
The use of whole serum makes it possible to study several APPs simultaneously, but seems to impair the resolution of SAA isoforms, since only two different human SAA isoforms have been found when acute phase whole serum has been used (Bini et al., 1996), whereas the studies based on protein material eluted from HIC have revealed five human isoforms (Foyn Bruun et al., 1995). An extension to the identification of isoforms with 2D electrophoresis is use of the technique to examine whether there are individual patterns following different acute phase stimuli and/or different dynamics over time of the isoforms. In one report using 2D electrophoresis to investigate murine SAA isoform patterns following stimulation with lipopolysacharide (LPS), different cytokines and combinations of cytokines and dexamethasone, similar patterns with respect to the relative amount of the different isoforms were seen after the different stimuli (Foyn Bruun et al., 1998). Furthermore, SAA from human patients with different diseases exhibited similar isoform patterns using electrofocusing followed by immunoblotting (Maury et al., 1985). In contrast, investigators using HIC and

electrofocusing to isolate isoforms of human SAA in serum reported differential dynamics over time (Raynes & McAdam, 1991). Studies of the dynamics of equine SAA isoforms have not been performed except for the small material of horses investigated in our paper, where no differences in isoform patterns were found between horses suffering from different diseases. The drawback of the 2D electrophoresis method when studying dynamics of SAA isoforms is its relative insensitivity to quantitative changes, an advantage might be the high resolution and easy identification of the spots representing SAA.

Characteristics of the immunoassay for measurement of equine SAA (paper II)

When purified equine SAA was used as the primary standard, a calibration curve ranging from 0.05 to 105 mg/L was produced. This standard curve was used to calibrate the working standard, which after dilution to suitable concentrations generated a standard curve in the range of 0.02-24.2 mg/L. The detection limit of the assay was 0.01 mg/L. Taking into account the uniform sample dilution of 1:50 and the coefficients of variation, an overall working range of 3-1210 mg/L was set. In this interval, the intra-assay CV was <10%.

The affinity-purified antibody used had similar affinity to the three acute phase SAA isoforms as tested by Western blot. No non-specific binding was detected in the blots.

Purification of SAA involves delipidation of the protein by dissociation from the lipoprotein complex in which SAA is transported. From an assay-development-point of view this is troublesome since the SAA to be measured in the sample is still complexed with HDL. In assays using purified SAA as a standard, denaturation and delipidation have been used to bring the SAA in the sample to the same form as in the standard solution. We chose instead to use an acute phase serum calibrated against purified SAA as a standard, and thereby avoided consumption of purified SAA. A shift towards higher signals was noted for the native SAA standard compared with purified SAA. This has previously been noted by Godenir and co-authors (1985), who investigated the shift of standard curves due to differing immunoreactivity using purified and native SAA and AA. The different properties of HDL-bound and purified lipid-free SAA were also made apparent by the lack of a parallelism between primary (purified SAA) and working (native SAA) standard curves in our system. In contrast to these findings and in spite of our and several other reports on lack of agreement between displacement curves for purified and native SAA, recent studies on the antigenic determinants of purified SAA using sequence-specific antibodies indicate that the accessible antigenic determinants are the same in both types of SAA (Malle et al., 1995; Malle et al., 1998). The authors cited draw the conclusion that purified SAA would be suitable as standard for native SAA due to the antigenic similarities between them. However, we did not find the two types of SAA entirely comparable in our system. Therefore, the working standard was calibrated against the primary standard in the interval of the primary standard

curve where the agreement between the curves was best. Since increasing dilution factors were noted to give increasing absolute SAA concentrations, only one sample dilution was used to further standardise the assay procedure. The problem of discrepancy between absolute SAA concentrations calculated after assay of the same sample at different dilutions was recently noted in a study evaluating external calibration of human SAA methods with a WHO standard (Poole et al., 1998). A recent approach to the question of agreement between purified standard and native samples was taken by McCormack and co-authors (1996), who used recombinant SAA to more easily be able to produce sufficient amounts of standard and utilised a sample buffer containing propan-2-ol to achieve dissociation of SAA from the lipoprotein complex. This approach rendered similar dilution profiles between purified SAA standard and propan-2-ol-treated samples.

In the previously published EIA for equine SAA (Satoh et al., 1995) a method of standardisation similar to ours is used although SAA is purified by repeated gel filtration. This EIA has a standard curve range of 0.02-5 mg/L and combined with a uniform sample dilution of 1:200, the working range 4-1000 mg/L is obtained. This is similar to the working range of our assay. Earlier methods for measurement of equine SAA include an electroimmunoassay (rocket electrophoresis) (Pepys et al., 1989) and a RID assay (Nunokawa et al., 1993). The electroimmunoassay has a range of 1-50 U/L and assays undiluted samples. It is calibrated using an acute phase serum, whose SAA concentration was assigned arbitrarily. Samples above 50 U/L are reassayed using a sample dilution of 1:10. The arbitrary units used correspond roughly to mg/L, according to the authors. The working range of the RID method is 5-160 mg/L and undiluted samples are used. The method is calibrated using SAA purified by repeated gel filtration. The main drawback of the electroimmunoassay and the RID assay is that they are time consuming and rather laborious to perform. The completion time of the EIA developed by Satoh and co-authors (1995), on the other hand, is comparable to ours.

Different methods to determine the SAA concentration of the purified SAA standard are employed for the assays described above. For the RID method the purified and freeze-dried SAA was weighed and diluted to suitable concentrations. In our hands, weighing of the standard has given considerably higher values compared with determination of the SAA concentration by amino acid composition analysis, even when the SAA is lyophilised to apparent dryness (unpublished observations). Likewise, using the biochemical Lowry method to determine protein concentration of the SAA standard, as in the SAA EIA described above, also gives higher concentrations compared with amino acid analysis (Marhaug, 1983). In addition, SAA purified by repeated gel filtration might contain other proteins (Marhaug & Husby, 1981). These differences can partly explain the differences seen between SAA concentrations determined by our method and those determined by the previous methods (see below).

Clinical evaluation of SAA measurements in the horse (Papers II-V)

SAA concentrations in healthy horses (paper II)

The reference interval for SAA concentration in healthy, adult horses was <7 mg/L. Previously published reference ranges for adult horses are 2-41 mg/L (RID) (Nunokawa et al., 1993) and <33 mg/L (EIA) (Satoh et al., 1995), calculated on the same set of horses. The discrepancy between our interval and the others is probably due in part to the difference in standardisation and design of the methods and to the fact that different methods were used for determining the protein concentration in the standard.

Non-infectious inflammation (paper II-III)

After surgical trauma (castration) the SAA concentrations in serum increased quickly and reached maximal levels on day 2 after surgery, whereupon they approximately halved each day to reach baseline concentration again within a week. Maximal concentrations (10-110 mg/L) differed considerably between individuals, whereas the dynamics over time were consistent. The SAA response seen in our investigation is in accordance with previous studies of the SAA response after surgery on healthy horses (Pepys et al., 1989; Satoh et al., 1995). Satoh and co-authors (1995) also report the SAA response after bowel surgery (jejunojejunostomy) on a healthy horse. In that case, the maximal SAA concentration was higher than after castration and persisted until day 7 after surgery. The reason for this extended response was probably the more extensive tissue damage compared with castration.

In addition to SAA, total white blood cell count and fibrinogen were analysed on the castrated horses in our study. These parameters remained within reference limits or were only slightly elevated, and there were no systemic clinical signs of inflammation (unpublished observations), which demonstrates that SAA is a sensitive marker of inflammation caused by surgical trauma. Our results concerning the normal SAA response following surgery indicate that SAA analyses could be used for monitoring post-surgical recovery and for detecting systemic post-surgical complications in the horse.

In humans, post-surgical SAA measurements have been used to monitor rejection episodes after kidney, liver and heart transplantation. The SAA response to the surgical treatment itself in these cases has been reported as having dynamics comparable to those seen after surgery in the horse, with maximal values on day 2 and regained baseline values within a week (Maury et al., 1983; Feussner et al., 1994; Fukuda et al., 1998). In these investigations, viral post-surgical infections gave rise to increased SAA concentrations as well.

In our study, non-infectious inflammation was further evaluated by investigating the SAA response during experimental aseptic arthritis (amphotericin B-induced synovitis/capsulitis). A similar response as following surgery was seen, with

maximal concentrations on day 2 pi, a wide range of maximal concentrations (46-286 mg/L) and a return to pre-induction values at day 15 pi. In addition to SAA; haptoglobin, fibrinogen and α_2-globulins were measured during the course of the aseptic arthritis in the joint. These proteins increased only mildly, the relative maximal increases being 55% for α_2-globulins, 87% for fibrinogen and 114% for haptoglobin. The relative maximal increase of SAA, on the other hand, was 227 times the pre-induction value. All proteins had returned to pre-induction values by day 15 pi.

The similarities between the responses after surgery and aseptic arthritis were probably due to the similarity of the stimuli, including aseptic tissue damage and distinct onset and termination of the stimulus. As a model for aseptic inflammation, the induction of arthritis with amphotericin B could be compared to the induction of aseptic inflammation by intramuscular or subcutaneous injection of irritating substances such as turpentine and casein. Turpentine injection has been used to induce the APP response in several species including horse (Nunokawa et al., 1993), pig (González-Ramón et al., 1995; Eckersall et al., 1996) and dog (Yamashita et al., 1994). In the cited studies, SAA and other major APPs comparable to SAA were measured, including CRP and major acute phase protein of pigs (pig-MAP). The dynamics of SAA, CRP and pig-MAP in these instances were similar to the responses seen during aseptic inflammation described in our study.

Apart from looking at aseptic arthritis as a model for non-infectious inflammation in the horse, the results from the study presented in paper III demonstrate that this type of non-infectious arthritis induces release of cytokines, which elicit a systemic APR in the horse. This systemic effect of the cytokines produced in the inflamed joint has attracted little or no attention in equine research although many authors have addressed the intra-articular induction of cytokine release in aseptic arthritis. IL-1, IL-6 and TNF-α - the main inducers of the APP synthesis - have been shown to be present in synovial fluid during arthritis in the horse (Alwan et al., 1991; Hawkins et al., 1993).

Our finding, that an aseptic arthritis in a single joint can elicit an APR measurable as prominent increases of a major APP can be important clinically when differentiating between different types of joint disease. Increased APP levels need not be indicative of an infection.

In humans, elevated APP levels have been recorded in connection with osteoarthritis (Spector et al., 1997), which is also commonly encountered in equine practice as a sequel to several types of joint disease. Serum markers for osteoarthritis and degenerative joint disease have been sought also in equine research but these investigations have been focused on measuring products from cartilage metabolism, such as keratan sulfate. The results of these studies have been somewhat contradictory (Todhunter et al., 1997; Okumura et al., 1998). Possibly such studies should be extended to include also the APPs since cytokine production has been detected in osteoarthritic joint disease in the horse (Morris et al., 1990; Billinghurst et al., 1995). However, this field of application for APPs

would probably call for assay methods for equine SAA which are more sensitive than those used at present, since the inflammatory process is mild and only minor elevations of SAA concentrations could be expected. The unspecific nature of the APR should also be borne in mind.

Infectious disease

Viral infection (paper IV)

Naturally occurring acute equine influenza A2 virus infection elicited an APR measurable as increased concentrations of SAA in the acute stage of the disease. The horses were sampled within the first 48 hours of clinical signs. Acute stage SAA concentrations were significantly higher in horses sampled late in the acute stage than in those sampled early. This indicates that the SAA concentration increased during the first 48 hours of clinical signs, which is in agreement with the dynamics seen in cases of aseptic inflammation. In the convalescent stage, the SAA concentrations could not be distinguished from those of healthy horses. Horses in which viral infection could be detected in nasal secretions at the acute stage sampling had higher SAA concentrations than horses that were virus positive later in the acute stage or virus negative in nasal secretions throughout the acute stage.

During experimental influenza virus infection in humans dramatically increased SAA concentrations were seen during the first 48 hours of clinical signs (Whicher et al., 1985). The only reports on SAA responses in viral infection in the horse deal with experimental EHV-1 infection in pregnant mares (Pepys et al., 1989) and one foal suffering from intra-uterine EHV-1 infection (Chavatte et al., 1991). Pepys and co-authors (1989) reported slightly elevated SAA concentrations in three race horses with clinical signs of respiratory disease that seroconverted to EHV-1. Following experimental infection with EHV-1, the SAA concentrations of the mares increased rapidly to reach levels a hundredfold to several hundredfold that of baseline approximately 2-4 days post-challange. The mares regained baseline concentrations within 2 weeks. In our study, the vast majority of the horses had normal SAA concentrations in the convalescent stage. They were sampled once between 11 and 22 days after the acute stage sample was taken. This indicates that the SAA response during uncomplicated influenza virus infection is shorter than 2-3 weeks, and thus resembles the response following experimental EHV-1 infection.

Studies on viral infection in human patients, which determined SAA concentrations in paired samples (acute and convalescent stage) have revealed dynamics of the SAA response similar to those outlined in our study during influenza virus infection as well as during several other viral infections (Miwata et al., 1993; Nakayama et al., 1993).

In conclusion, our study demonstrate that SAA responds to equine influenza virus infection and previous studies have shown that equine SAA responds to EHV-1 infection. It should be remembered, however, that SAA is not a specific marker of viral infection.

Infections in foals (paper V)

Foals with bacterial infections (n=8) had higher SAA and fibrinogen concentrations on admission to the clinic than foals with non-bacterial or uncertain diagnoses (n=17), whereas there was no statistical difference in total leukocyte and neutrophil counts between the two groups.

On admission, neonatally weak foals with negative blood cultures had normal SAA and fibrinogen concentrations and varying leukocyte and neutrophil counts. The foals with positive blood cultures, that were euthanised, had markedly increased SAA, decreased or increased fibrinogen concentration and a leukogram showing leukopenia and neutropenia with toxic changes in the neutrophils. Foals with ambiguous blood cultures had moderately to markedly increased SAA concentrations, and normal fibrinogen concentrations, leukocyte and neutrophil counts on admission. All foals with negative or ambiguous blood cultures recovered and had normal or decreasing SAA concentration on discharge. The foal born with EHV-1 infection, and which subsequently died, had moderately increased SAA, normal fibrinogen concentration and leuko- and neutropenia on admission. Foals with *Rhodococcus equi*-pneumonia had increased concentrations of SAA and fibrinogen, leukocytosis and neutropenia on admission. On discharge, recovered foals (n=3) had normal SAA concentrations, whereas fibrinogen and leukocyte counts and neutrophil counts were still increased. In foals that were euthanised due to lack of response to treatment and poor prognosis (n=2) or died (n=1), leukocyte and neutrophil counts, SAA and fibrinogen were elevated in the last sample taken prior to death, except in one foal, euthanised due to cardiac arrhythmias, which had normal SAA concentration.

There were no consistent changes indicating inflammation in the parameters measured in diarrhoeic foals and there was no statistical difference between rotavirus positive (n=4) and rotavirus negative (n=6) foals in this respect.

The finding in our study, that SAA concentrations are higher in foals with bacterial infections than in foals with non-bacterial or uncertain diagnoses, is partly supporting the results of Chavatte and co-workers (1991). They found significantly higher SAA concentrations in a group of foals with verified and suspected bacterial and viral infections compared to young foals presenting other diseases not diagnosed as infections, and also compared to healthy foals. In our investigation, all but one of the foals with viral infections included, suffered from rotavirus infections, which, in general, did not mount a notable SAA response. However, SAA responds prominently to systemic viral infection (paper IV and Pepys et al., 1989). Therefore, it can not be expected, in spite of the results of our study, that it will be generally possible to distinguish between viral and bacterial infections by measuring SAA.

However, in the case of neonatal weakness, the main issue is whether the foal has a bacterial infection or if other reasons for the weakness should be sought. In this setting, it seems from our study and others that SAA measurements might have

some advantages compared to fibrinogen. Firstly, interpretation of fibrinogen values from foals with presumed septicaemia could be complicated by the fact that falsely low concentrations might occur due to a consumption coagulopathy, which can be seen in connection with sepsis. Although the consumption of fibrinogen seldom is extensive enough to cause hypofibrinogenaemia (Duncan & Prasse, 1986; Barton et al., 1998), it could be speculated that the very young foal would be more prone to develop hypofibrinogenaemia, since young foals have been shown to have lower fibrinogen concentrations than older foals and adult horses (Barton et al., 1995). Secondly, SAA seems to respond quicker and more prominently to inflammatory stimuli compared to fibrinogen (paper III; Paradis, 1994), which reasonably means that an infection can be detected earlier with SAA measurements. Thirdly, relative increases in fibrinogen concentrations can occur due to haemoconcentration/dehydration, and this may render falsely high concentrations in diseased foals (paper III; Duncan & Prasse, 1986; Robinson et al., 1993). Due to the very low SAA concentrations in healthy horses and the prominent increases during inflammation, the interpretation of SAA values is not hampered by dehydration (paper III). One possible disadvantage of SAA when assessing bacterial infection is that the SAA response is non-specific. Therefore combination of SAA measurements with leukocyte counts and leukocyte morphology will probably add to the diagnostic value of SAA when trying to differentiate bacterial infection from other diseases. Another disadvantage of SAA when used in intensive care at present is the more time-consuming assays necessary for analysis compared to fibrinogen, since short analysis time is important in the management of critically ill patients.

The importance of adequate treatment for the outcome of *Rhodococcus equi*-pneumonia in the foal has been made clear in previous studies (Sweeney et al., 1987; Ainsworth et al., 1998). Our study is far to small and brief to determine the usefulness of SAA as a tool for monitoring treatment of *Rhodococcus equi*-pneumonia, and the dynamics of this marker in relation to clinical status. However, it seems that SAA responds rapidly to treatment and that decreasing concentrations possibly reflect declining disease activity and spread of the infection. On the other hand,our study indicates that even if the systemic inflammatory effects of the infection are suppressed by the treatment, as illustrated by decreasing SAA concentrations, the pneumonic lesions may be of such magnitude that fatal disturbances on the respiratory and circulatory systems may occur in late stages of the disease. This has been further illustrated in a previous study, in which it was reported, that factors associated with non-survival in *Rhodococcus equi*-pneumonia included circulatory and respiratory distress and severe lung abnormalities, whereas haematological parameters and fibrinogen concentration did not differ between survivors and non-survivors (Ainsworth et al., 1998).

Although not addressed in our study, it could be speculated that SAA measurements might be an aid in the early detection of foals with incipient *Rhodococcus equi*-pneumonia. The basis for this speculation is the narrow

reference range and low concentrations of SAA in healthy horsescompared to leukocyte counts and fibrinogen concentration, and the assumption that increased SAA concentrations would occur prior to prominent clinical signs of pneumonia and/or radiographical changes.

The changes in leukocyte and neutrophil counts as well as in fibrinogen and SAA concentrations were inconsistent and mild both in rotavirus positive and negative foals with diarrhoea. In rotavirus infected foals, this finding indicates that if an acute phase response was evoked by the infection, it was too mild or transient to be recorded as changed values of the parameters measured. In rotavirus negative foals the conclusion must be that the cause of the diarrhoea was non-inflammatory or that the foals had an undetected infection with an influence on the parameters measured similar to that of rotavirus.

Conclusions

- Equine SAA can be purified using a method based on hydrophobic interaction chromatography, gel filtration and cation exchange chromatography.

- Equine SAA exhibits three circulating acute phase isoforms with isoelectric points of 8.0, 9.0 and 9.7 and with differences in their amino acid sequence at positions 16, 44 and 59. These isoforms can be clearly separated using two-dimensional electrophoresis.

- Equine SAA can be measured quantitatively in serum using an acute phase serum calibrated against purified SAA as standard and an affinity-purified anti-equine amyloid A antibody. This antibody binds all three acute phase isoforms of SAA equally.

- Clinically healthy, adult horses have low SAA concentrations (below 7 mg/L).

- Surgical trauma and experimental non-infectious arthritis trigger prominent SAA responses characterised by maximal SAA concentrations after 36-48 hours. After 48 hours the SAA concentrations decrease rapidly and reach normal concentrations again within 1-2 weeks.

- Haptoglobin, fibrinogen and α_2-globulins increases mildly and reaches maximal concentrations later compared to SAA during experimental non-infectious arthritis.

- Equine influenza virus infection elicits increasing SAA concentrations within the first 48 hours of clinical signs. In the convalescent stage, 2-3 weeks after the first appearance of clinical signs, the SAA concentrations are normalised.

- SAA concentrations are significantly higher in foals with verified bacterial infections than in foals with non-bacterial illness.

- SAA concentrations were markedly to moderately increased in neonatally weak foals with verified septicaemia (positive blood culture) and in neonatally weak foals with ambiguous blood cultures, whereas neonatally weak foals with negative blood cultures had normal SAA concentrations.

- *Rhodococcus equi*-pneumonia elicited increased SAA concentrations, which decreased during treatment.

- SAA concentration, fibrinogen concentration and leukocyte and neutrophil count were generally within reference ranges on admission and during treatment of rotavirus negative and rotavirus positive foals with diarrhoea.

Is SAA a useful marker of inflammation in the horse?

In the investigations presented in this thesis it is demonstrated that equine SAA is a sensitive marker of inflammation. Following the results presented above, some areas of use can be outlined:

- Due to its sensitivity, equine SAA is well suited to areas of equine research, where detailed studies of inflammatory processes or infections are needed.

- Equine SAA responds more prominently to tissue damage and infection, than any of the presently available non-specific markers that were investigated in this work. Therefore SAA measurements increase the possibilities of detecting inflammatory processes and of distinguishing them from non-inflammatory disorders.

- Equine SAA responds rapidly to surgical trauma and its dynamics after surgery are consistent over time. SAA measurements can therefore aid in the detection of post-surgical complications in the horse.

- Equine SAA decreased rapidly in response to efficient antimicrobial therapy in *Rhodococcus equi*-pneumonia. Although based on only a few horses, this finding suggests that SAA measurements might be used as an aid when monitoring response to treatment.

- Our findings in neonatally weak foals suggest that SAA measurements might be helpful in differentiating between foals with septicaemia and foals weak for other reasons.

When using SAA as a marker of inflammation, the following facts should be borne in mind:

- The non-specific nature of the SAA response. Increased SAA concentrations do not give information on the cause of the disorder detected. The SAA concentration reflects disease activity rather than type of disease.

- There is substantial variation between subjects regarding the magnitude of the concentration increase of SAA following a uniform stimulus.

- Serial measurements are preferrable to single measurements, since serial measurements give the opportunity to relate decreasing or increasing SAA

concentrations to the course of the disease encountered, to recovery or deterioration of the patient or to response to treatment.

In conclusion, measurements of equine SAA have the potential of being useful in both clinical practice and research in the areas outlined above.

References

Agrawal A, Kilpatrick JM & Volanakis JE, 1993. Structure and function of human C-reactive protein. In *Acute Phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications.* Eds. A Mackiewicz, I Kushner & H Baumann, CRC Press, Boca Raton, Florida, pp 79-92.

Ainsworth DM, Eicker SW, Yeagar AE, Sweeney CR, Viel L, Tesarowski D, Lavoie J-P, Hoffman A, Paradis MR, Reed SM, Erb HN, Davidow E & Nalevanko M, 1998.

Allen BV & Archer RK, 1971. Haptoglobins in the horse. *Vet Rec* 89: 106-109.

Alsemgeest SP, Horadagoda A, Hulskamp-Koch CK, Tooten PC, Kim DH, Niewold TA & Gruys E, 1995a. First evidence for the existence of multiple isoforms of bovine serum amyloid-A (apoSAA). *Scand J Immunol* 41: 407-413.

Alsemgeest SPM, Jonker FH, Taverne MAM, Kalsbeek HC, Wensing T & Gruys E, 1995b. Serum amyloid-A (SAA) and haptoglobin (Hp) plasma concentrations in newborn calves. *Theriogenology* 43: 381-387.

Alsemgeest SPM, Kalsbeek HC, Wensing T, Koeman JP, van Ederen AM & Gruys E, 1994. Concentrations of serum amyloid-A (SAA) and haptoglobin (Hp) as parameters of inflammatory diseases in cattle. *Vet Q* 16: 21-23.

Alsemgeest SPM, Lambooy IE, Wierenga HK, Dieleman SJ, Meerkerk B, van Ederen AM & Niewold TA, 1995c. Influence of physical stress on the plasma concentration of serum amyloid-A (SAA) and haptoglobin (Hp) in calves. *Vet Q* 17: 9-12.

Alsemgeest SPM, Taverne MAM, Boosman R, van der Weiden BC & Gruys E, 1993. Peripartum acute-phase protein serum amyloid-A concentration in plasma of cows and fetuses. *Am J Vet Res* 54: 164-167.

Alwan WH, Carter SD, Dixon JB, Bennett D, May SA & Edwards GB, 1991. Interleukin-1-like activity in synovial fluids and sera of horses with arthritis. *Res Vet Sci* 51: 72-77.

Anders RF, Natvig JB, Michaelsen TE & Husby G, 1975. Isolation and characterization of amyloid-related serum protein SAA as a low molecular weight protein. *Scand J Immunol* 4: 397-401.

Andreotti F, Burzotta F & Maseri, 1999. Fibrinogen as a marker of inflammation: a clinical view. *Blood Coagul Fibrinolysis* 10, Suppl 1: S3-S4.

Andrews DA, Reagan WJ & DeNicola DB, 1994. Plasma fibrinogen in recognizing equine inflammatory disease. *Comp Continuing Education* 16: 1349-1354.

Badolato R, Wang JM, Murphy WJ, Lloyd AR, Michiel DF, Bausserman LL, Kelvin DJ & Oppenheim JJ, 1994. Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. *J Exp Med* 180: 203-209.

Baltz ML, De Beer FC, Feinstein A, Munn EA, Milstein CP, Fletcher TC, March JF, Taylor J, Bruton C, Clamp JR, Davies AJ & Pepys MB, 1982. Phylogenetic aspects of C-reactive protein and related proteins. *Ann N Y Acad Sci* 389: 49-75.

Baltz ML, Rowe IF, Caspi D, Turnell WG & Pepys MB, 1987. Acute-phase high-density lipoprotein in the rat does not contain serum amyloid A protein. *Biochem J* 242: 301-303.

Barton MH, Morris DD, Norton N & Prasse KW, 1998. Hemostatic and fibrinolytic indices in neonatal foals with presumed septicemia. *J Vet Intern Med* 12: 26-35.

Barton MH, Morris DD, Crowe N, Collatos C & Prasse KW, 1995. Hemostatic indices in healthy foals from birth to one month of age. *J Vet Diagn Invest* 7: 380-385.

Beach CM, De Beer MC, Sipe JD, Loose LD & De Beer FC, 1992. Human serum amyloid A protein. Complete amino acid sequence of a new variant. *Biochem J* 282: 615-620.

Becker U, Bartl K & Whalefeld A, 1984. A functional photometric assay for plasma fibrinogen. *Thromb Res* 35: 475-484.

Benditt EP & Eriksen N, 1977. Amyloid protein SAA is associated with high density lipoprotein from human serum. *Proc Natl Acad Sci USA* 74: 4025-4028.

Benditt EP & Eriksen N, 1984.

Benditt EP, Eriksen N & Hanson RH, 1979. Amyloid protein SAA is an apoprotein of mouse plasma high density lipoprotein. *Proc Natl Acad Sci USA* 76: 4092-4096.

Betts JC, Edbrooke MR, Thakker RV & Woo P, 1991. The human acute phase serum amyloid A gene family: Structure, evolution and expression in hepatoma cells. *Scand J Immunol* 34: 471-482.

Billinghurst RC, Fretz PB & Gordon JR, 1995. Induction of intra-articular tumour necrosis factor during acute inflammatory responses in equine arthritis. *Equine vet J* 27: 208-216.

Bini L, Magi B, Marzocchi B, Cellesi C, Berti B, Raggiaschi R, Rossolini A & Pallini V, 1996. Two-dimensional electrophoretic patterns of acute-phase human serum proteins in the course of bacterial and viral diseases. *Electrophoresis* 17: 612-616.

Biran H, Friedman N, Neumann L, Pras M, Shainkin-Kestenbaum R, 1986. Serum amyloid A (SAA) variations in patients with cancer: correlation with disease activity, stage, primary site and prognosis. *J Clin Pathol* 39: 794-797.

Boosman R, Niewold TA, Mutsaers CWAAM & Gruys E, 1989. Serum amyloid A concentrations in cows given endotoxin as an acute-phase stimulant. *Am J Vet Res* 50: 1690-1694.

Butler A & Whitehead AS, 1997. Structure of the mouse serum amyloid A 5 (SAA5) gene: relationship to other members of the serum amyloid A family. *Scand J Immunol* 45: 160-165.

Časl M-T & Grubb A, 1993. A rapid enzyme-linked immunosorbent assay for serum amyloid A using sequence-specific antibodies.*Ann Clin Biochem* 30: 278-286.

Časl M-T, Surina B, Glojnaric-Spasic I, Pape E, Jagarinec N & Kranjcevic S, 1995. Serum amyloid A protein in patients with acute myocardial infarction. *Ann Clin Biochem* 32: 196-200.

Caspi D, Snel FWJJ, Batt RM, Bennett D, Rutteman GR, Hartman EG, Baltz ML, Gruys E & Pepys MB, 1987. C-reactive protein in dogs. *Am J Vet Res* 48: 919-921.

Chavatte PM, Pepys MB, Roberts B, Ousley JC, McGladdery AJ & Rossdale PD, 1991. Measurement of serum amyloid A protein (SAA) as an aid to differential diagnosis of infection in newborn foals. In *Equine infectious diseases Vol VI.* Eds. W Plowright, PD Rossdale & JF Wade, R & W Publications, Newmarket, UK, pp 33-38.

Clifton PM, Mackinnon AM & Barter PJ, 1985. Effects of serum amyloid A protein (SAA) on composition, size, and density of high density lipoprotein in subjects with myocardial infarction. *J Lipid Res* 26: 1389-1398.

Conner JG, Eckersall PD, Wiseman A, Aitchison TC & Douglas TA, 1988. Bovine acute phase response following turpentine injection. *Res Vet Sci* 44: 82-88.

De Beer MC, Beach CM, Shedlovsky SI & De Beer FC, 1991. Identification of a novel serum amyloid A protein in BALB/c mice. *Biochem J* 280: 45-49.

De Beer MC, De Beer FC, Beach CM, Carreras I & Sipe JD, 1992. Mouse serum amyloid A protein. Complete amino acid sequence and mRNA analysis of a new isoform. *Biochem J* 283: 673-678.

DiBartola SP, Reiter JA, Cornacoff JB, Kociba GJ & Benson MD, 1989. Serum amyloid A protein concentration measured by radial immunodiffusion in Abyssinian and non-Abyssinian cats. *Am J Vet Res* 50: 1414-1417.

Dillman RC & Coles EH, 1966. A canine serum fraction analogous to human C-reactive protein. *Am J Vet Res* 27: 1769-1775.

Dobryszycka W, 1993. Haptoglobin: Retrospectives and perspectives. In *Acute Phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications.* Eds. A Mackiewicz, I Kushner & H Baumann, CRC Press, Boca Raton, Florida, pp 185-206.

Doherty NS, Littman BH, Reilly K, Swindell AC, Buss JM & Anderson NL, 1998. Analysis of changes in acute-phase plasma proteins in an acute inflammatory response and in rheumatoid arthritis using two-dimensional gel electrophoresis. *Electrophoresis*19: 355-363.

Dubois DY & Malmendier CL, 1988. Non-competitive enzyme-linked immunosorbent assay for human apolipoprotein SAA or S. *J Immunol Methods* 112: 71-75.

Duncan JR & Prasse KW, 1986. Proteins, lipids and carbohydrates. In *Veterinary Laboratory Medicine, 2nd Ed,* Iowa State University Press, Ames, Iowa, pp 105-120.

Dwulet FE, Wallace DK & Benson MD, 1988. Amino acid structures of multiple forms of amyloid-related serum protein SAA from a single individual. *Biochemistry* 27: 1677-1682.

Eckersall PD, 1992. Animal acute phase protein; past, present and future. *Proc Vth Congress of ISACB*, 2-6 September, Parma, Italy, 97-103.

Eckersall PD, Duthie S, Toussaint JM, Gruys E, Heegaard P, Alava M, Lipperheide C & Madec F, 1999. Standardisation of diagnostic assays for animal acute phase proteins. *Adv Vet Med* 41: 643-655.

Eckersall PD, Saini PK & McComb C, 1996. The acute phase response of acid soluble glycoprotein, α_1-acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein, in the pig. *Vet Immunol Immunopathol* 51: 377-385.

Elson EC, 1974. Quantitative determination of serum haptoglobin. A simple and rapid method. *Am J Clin Pathol* 62: 655-663.

Ericsson LH, Eriksen N, Walsh KA & Benditt EP, 1987. Primary structure of duck amyloid protein A. The form deposited in tissues may be identical to its serum precursor. *FEBS Lett* 218: 11-16.

Eriksen N & Benditt EP, 1980. Isolation and characterization of the amyloid-related apoprotein (SAA) from human high density lipoprotein. *Proc Natl Acad Sci USA* 77: 6860-6864.

Eriksen N & Benditt EP, 1984. Trauma, high density lipoproteins, and serum amyloid protein A. *Clin Chim Acta* 140: 139-149.

Eriksson K-O, 1989. Hydrophobic interaction chromatography. In *Protein Purification. Principles, High Resolution Methods and Applications.* Eds J-C Janson & L Rydén, VCH Publishers, New York, NY, pp 207-226.

Fåhraeus R, 1921. The suspension stability of the blood. *Acta Med Scand* 55: 1-228.

Feussner G, Stech C, Dobmeyer J, Schaefer H, Otto G & Ziegler R, 1994. Serum amyloid A protein (SAA): a marker for liver allograft rejection in humans. *Clin Investig* 72: 1007-1011.

Foyn Bruun C, Nordstoga K, Sletten K, Husby G & Marhaug G, 1995. Serum amyloid A protein in humans and four animal species: a comparison by two dimensional electrophoresis. *Comp Biochem Physiol* 112B: 227-234.

Foyn Bruun C, Rygg M, Nordstoga K, Sletten K & Marhaug G, 1994. Serum amyloid A protein in mink during endotoxin induced inflammation and amyloidogenesis. *Scand J Immunol* 40: 337-344.

Foyn Bruun C, Sletten K, Husby G & Marhaug G, 1993. Characterization and quantification of mink serum amyloid A protein using two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis*14: 1372-1374.

Foyn Bruun C, Sletten K & Marhaug G, 1998. Mouse serum amyloid A (SAA) proteins isolated by two-dimensional electrophoresis: characterization of isotypes and the effect of separate and combined administrations of cytokines, dexamethasone and lipopolysacharide (LPS) on serum levels and isotype distribution. *Clin Exp Immunol* 111: 231-236.

Fukuda Y, Kanbe M, Sumimoto R, Yoneya T, Takeshita T, Hoshino S, Shintaku S & Dohi K, 1998. Examination of serum amyloid A protein in kidney transplant patients - comparison of serum amyloid A and C-reactive protein for monitoring occurrence of renal-allograft-related complications. *Hiroshima J Med Sci* 47: 63-67.

Fuller GM, 1993. Fibrinogen: A multifunctional acute phase protein. In *Acute Phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications.* Eds. A Mackiewicz, I Kushner & H Baumann, CRC Press, Boca Raton, Florida. pp 169-183.

Gabay C & Kushner I, 1999. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340: 448-454.

Gallin JI, Goldstein IM & Snyderman R, 1992. Overview. In *Inflammation: Basic principles and Clinical Correlates. 2nd Ed.* Eds: JI Gallin, IM Goldstein & R Snyderman, Raven Press, New York, NY, pp 1-5.

Ganroth K, 1973. Plasma protein response in experimental inflammation in the dog. *Res exp Med* 161: 251-261.

Gatt ME, Urieli-Shoval S, Preciado-Patt L, Fridkin M, Calco S Azar Y & Matzner Y, 1998. Effect of serum amyloid A on selected in vitro functions of isolated human neutrophils. *J Lab Clin Med* 132: 414-420.

Gervais C & Suh M, 1990. Serum amyloid A protein-related mRNA expression in herpes simplex virus type 2-transformed hamster cells. *Mol Cell Biol* 10: 4412-4414.

Giclas PC, Manthei U & Strunk RC, 1985. The acute phase response of C3, C5, ceruloplasmin, and C-reactive protein induced by turpentine pleurisy in the rabbit. *Am J Pathol* 120: 146-156.

Glitz D, 1992. Protein synthesis: translation and posttranslational modifications. In *Textbook of Biochemistry with Clinical Correlations*. Ed. TM Devlin, Wiley-Liss, New York, pp 723-766.

Godenir NL, Jeenah MS, Coetzee GA, Van der Westhuyzen DR Strachan AF & De Beer FC, 1985. Standardisation of the quantitation of serum amyloid A protein (SAA) in human serum. *J Immunol Methods* 83: 217-225.

González-Ramón N, Alava MA, Sarsa JA, Piñeiro M, Escartin A, Garcia-Gil A, Lampraeve F & Piñeiro A, 1995. The major acute phase serum protein in pigs is homologous to human plasma kallikrein sensitive PK-120. *FEBS Letters* 371: 227-230.

Gorevic PD, Rosenthal CJ & Frankilin EC, 1976. Amyloid-related serum component (SAA) - studies in acute infections, medullary thyroid carcinoma and postsurgery. *Clin Immunol Immunopathol* 6: 83-93.

Gouffaux M, Pastoret PP, Henroteaux M & Massip A, 1975. Feline infectious peritonitis: proteins of plasma and ascitic fluid. *Vet Pathol* 12: 335-348.

Gruys E, Obwolo MJ, Toussaint MJM, 1994. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. *Vet Bull* 64: 1009-1018.

Gruys E, Van Ederen AM, Alsemgeest SPM, Kalsbeek HC & Wensing T, 1993. Acute phase protein values in blood of cattle as indicator of animals with pathological processes. *Archiv für Lebensmittelhygiene* 44: 105-128.

Guo J-T, Aldrich CE, Mason WS & Pugh JC, 1996. Characterization of serum amyloid A protein mRNA expression and secondary amyloidosis in the domestic duck. *Proc Natl Acad Sci USA* 93:14548-14553.

Hachem H, Saïle R, Favre G, Bosco R, Raynal G, Fruchart JC & Soula G, 1991. A sensitive immunochemiluminescence assay for human serum amyloid A protein. *Clin Biochem* 24: 143-147.

Harvey JW, 1976. Quantitative determinations of normal horse, cat, and dog haptoglobins. *Theriogenology* 6: 133-138.

Hawkins DL, MacKay RJ, Gum GG, Colahan PT & Meyer JC, 1993. Effects of intra-articularly administered endotoxin on clinical signs of disease and synovial fluid tumor necrosis factor, interleukin 6, and prostaglandin E_2 values in horses. *Am J Vet Res* 54: 379-386.

Heegaard PMH, Klausen J, Nielsen JP, González-Ramón N, Piñeiro M, Lampraeve F & Alava MA, 1998. The porcine acute phase response to infection with *Actinobacillus pleuropneumoniae*. Haptoglobin, C-reactive protein, major acute phase protein and serum amyloid A protein are sensitive indicators of infection. *Comp Biochem Physiol* 119B: 365-373.

Hirvonen J, Eklund K, Teppo AM, Huszenicza G, Kulcsar M, Saloniemi H & Pyörälä S, 1999. Acute phase response in diary cows with experimentally induced *Escherichia coli* mastitis. *Acta Vet Scand* 40: 35-46.

Hocke G, Ebel H, Bittner K, Muller T, Kaffarnik H & Stainmetz A, 1989. A rapid laser immunonephelometric assay for serum amyloid A (SAA) and its application to the diagnosis of kidney allograft rejection. *Klin Wochenschr* 67: 447-451.

Hoffman JS, Ericsson LH, Eriksen N, Walsh KA & Benditt EP, 1984. Murine tissue amyloid protein AA. NH_2-terminal sequence identity with only one of two serum amyloid protein (apoSAA) gene products. *J Exp Med* 159: 641-646.

Horadagoda A, Eckersall PD, Alsemgeest SPM & Gibbs HA, 1993. Purification and quantitative measurement of bovine serum amyloid-A. *Res Vet Sci* 55: 317-325.

Horadagoda A, Eckersall PD, Hodgeson JC, Gibbs HA, Moon GM, 1994. Immediate responses in serum TNF_α and acute phase protein concentrations to infection with *Pasteurella haemolytica* A1 in calves. *Res Vet Sci* 57: 129-132.

Horadagoda NU, Knox KM, Gibbs HA, Reid SW, Horadagoda A, Edwards SE & Eckersall PD, 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Vet Rec* 144: 437-441.

Husby G, 1994. Classification of amyloidosis. *Bailliere's Clinical Rheumatology: Reactive Amyloidosis and the Acute Phase Response* 8: 503-511.

Husby G & Natvig JB, 1974. A serum component related to nonimmunoglobulin amyloid protein AS, a possible precursor of the fibrils. *J Clin Invest* 53: 1054-1061.

Husebekk A, Husby G, Sletten K, Marhaug G & Nordstoga K, 1986. Characterization of amyloid protein AA and its serum precursor SAA in the horse. *Scand J Immunol* 23: 703-709.

Husebekk A, Husby G, Sletten K, Skogen B & Nordstoga K, 1988. Characterization of bovine amyloid proteins SAA and AA. *Scand J Immunol* 27: 739-743.

Husebekk A, Skogen B, Husby G & Marhaug G, 1985. Transformation of amyloid precursor SAA to protein AA and incorporation in amyloid fibrils *in vivo*. *Scand J Immunol* 21: 283-287.

Hwang PK & Greer J, 1980. Interaction between hemoglobin subunits in the hemoglobin-haptoglobin complex. *J Biol Chem* 255: 3038-3041.

Jensen LE, Hiney MP, Shields DS, Uhlar CM, Lindsay AJ & Whitehead AS, 1997. Acute phase proteins in salmonids. Evolutionary analyses and acute phase response. *J Immunol* 158: 384-392.

Jensen LE & Whitehead AS, 1998. Regulation of serum amyloid A protein expression during the acute-phase response. *Biochem J* 334: 489-503.

Kajikawa T, Furuta A, Onishi T & Sugii S, 1996. Enzyme-linked immunosorbent assay for detection of feline serum amyloid A protein by use of immunological cross-reactivity of polyclonal anti-canine serum amyloid A protein antibody. *J Vet Med Sci* 58: 1141-1143.

Kajikawa T, Furuta A, Onishi T, Tajima T & Sugii S, 1999. Changes in concentrations of serum amyloid A protein, alpha 1-acid glycoprotein, haptoglobin, and C-reactive protein in feline sera due to induced inflammation and surgery. *Vet Immunol Immunopathol* 68: 91-98.

Kent JE & Goodall J, 1991. Assessment of an immunoturbidimetric method for measuring equine serum haptoglobin concentrations. *Equine Vet J* 23: 59-66.

Kisilevsky R, 1991. Serum amyloid A (SAA), a protein without a function: some suggestions with reference to cholesterol metabolism. *Med Hypotheses* 35: 337-341.

Kisilevsky R & Subrahmanyan L, 1992. Serum amyloid A changes high desity lipoprotein's cellular affinity. A clue to serum amyloid A's principal function. *Lab Invest* 66: 778-785.

Kisilevsky R & Young ID, 1994. Pathogenesis of amyloidosis. *Bailliere's Clinical Rheumatology: Reactive Amyloidosis and the Acute Phase Response* 8: 613-626.

Kluve-Beckerman B, Dwulet FE & Benson MD, 1988. Human serum amyloid A. Three hepatic mRNAs and the corresponding proteins in one person. *J Clin Invest* 82: 1670-1675.

Kluve-Beckerman B, Yamada T, Hardwick J, Liepnieks JJ & Benson MD, 1997. Differential plasma clearance of murine acute-phase serum amyloid A proteins SAA1 and SAA2. *Biochem J* 322: 663-669.

Koj A, Gauldie J & Baumann H, 1993. Biological perspectives of cytokine and hormone networks. In *Acute Phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications.* Eds: A Mackiewicz, I Kushner & H Baumann, CRC Press, Boca Raton, Florida, pp 275-287.

Kumon Y, Suehiro T, Hashimoto K, Nakatani K & Sipe JD, 1999. Local expression of acute phase serum amyloid A mRNA in rheumatoid arthritis synovial tissue and cells. *J Rheumatol* 26: 785-790.

Kushner I, 1982. The phenomenon of the acute phase response. *Ann N Y Acad Sci* 389: 39-48.

Kushner I & Mackiewicz A, 1993. The acute phase response: An overview. In *Acute Phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications.* Eds: A Mackiewicz, I Kushner & H Baumann, CRC Press, Boca Raton, Florida, pp 7-19.

Levartowsky D & Pras M, 1991. The effect of SAA-derived fragment - SAA_{2-82} - on platelet aggregation. In *Amyloid and Amyloidosis.* Eds: JB Natvig, Ø Førre, G Husby, A Husebekk, B Skogen, K Sletten & P Westermark, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 129-132.

Levin M, Pras M & Franklin EC, 1973. Immunologic studies of the major nonimmunoglobulin protein of amyloid. I. Identification and partial characterization of a related serum component. *J Exp Med* 138: 373-380.

Liepnieks JJ, Dwulet FE, Benson MD, Kluve-Beckerman B & Kushner I, 1991. The primary structure of serum amyloid A protein in the rabbit: Comparison with serum amyloid A proteins in other species. *J Lab Clin Med* 118: 570-575.

Linke RP, Bock V, Valet G & Rothe G, 1991. Inhibition of the oxidative burst response of N-formyl peptide-stimulated neutrophils by serum amyloid A protein. *Biochem Biophys Res Commun* 176: 1100-1105.

Lipperheide C, Diepers N, Lampreave F, Alava M & Petersen B, 1998. Nephelometric determination of haptoglobin plasma concentrations in fattening pigs. *J Vet Med A* 45: 543-550.

Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB & Maseri A, 1994. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med* 331: 417-424.

Lowell CA, Potter DA, Stearman RS & Morrow JF, 1986. Structure of the murine serum amyloid A gene family. Gene conversion. *J Biol Chem* 261: 8442-8452.

Malle E & De Beer FC, 1996. Human serum amyloid A (SAA) protein: a prominent acute -phase reactant for clinical practice. *Eur J Clin Invest* 26: 427-435.

Malle E, Herz R, Artl A, Ibovnik A, Andrae F & Sattler W, 1998. Mapping of antigenic determinants of purified, lipid-free human serum amyloid A proteins. *Scand J Immunol* 48: 557-561.

Malle E, Münscher G, Müller T, Vermeer H & Ibovnik A, 1995. Quantification and mapping of antigenic determinants of serum amyloid A (SAA) protein utilizing sequence-specific immunoglobulins and Eu^{3+} as a specific probe for time-resolved fluorometric immunoassay. *J Immunol Methods* 182: 131-144.

Marhaug G, 1983. Three assays for the characterization and quantitation of human serum amyloid A. *Scand J Immunol* 18: 329-338.

Marhaug G & Dowton SB, 1994. Serum amyloid A: An acute phase apolipoprotein and precursor of AA amyloid. *Bailliere's Clinical Rheumatology: Reactive Amyloidosis and the Acute Phase Response* 8: 553-573.

Marhaug G, Hackett B & Dowton SB, 1997. Serum amyloid A gene expression in rabbit, mink and mouse. *Clin Exp Immunol* 107: 425-434.

Marhaug G, Harklau L, Olsen B, Husby G, Husebekk A & Wang H, 1986. Serum amyloid A protein in acute myocardial infarction. *Acta Med Scand* 220: 303-306.

Marhaug G & Husby G, 1981. Characterization of human amyloid-related protein SAA as a polymorphic protein: association with albumin and prealbumin in serum. *Clin exp Immunol* 45: 97-106.

Marhaug G, Husby G & Dowton SB, 1990. Mink serum amyloid A protein. Expression and primary structure based on cDNA sequences. *J Biol Chem* 265: 10049-10054.

Marhaug G, Permin H & Husby G, 1983. Amyloid-related serum protein (SAA) as an indicator of lung infection in cystic fibrosis. *Acta Paediatr Scand* 72: 861-866.

Marhaug G, Sletten K & Husby G, 1982. Characterization of amyloid related protein SAA complexed with serum lipoproteins (apoSAA). *Clin exp Immunol* 50: 382-389.

Maudsley S, Rowe IF, De Beer FC, Munn EA, Herbert J Feinstein A & Pepys MB, 1987. Identification and isolation of two pentraxins from bovine serum. *Clin exp Immunol* 67: 662-673.

Maury CPJ, 1985. Comparative study of serum amyloid A protein and C-reactive protein in disease. *Clin Sci* 68: 233-238.

Maury CP, Ehnholm C & Lukka M, 1985. Serum amyloid A protein (SAA) subtypes in acute and chronic inflammatory conditions. *Ann Rheum Dis* 44: 711-715.

Maury CP, Teppo A, Eklund B & Ahonen J, 1983. Serum amyloid A protein: a sensitive indicator of renal allograft rejection in humans. *Transplantation* 36: 501-504.

McAdam KPWJ, Anders RF, Smith SR, Rusell DA & Price MA, 1975. Association of amyloidosis with erythema nodosum leprosum reactions and recurrent neutrophil leukocytosis in leprosy. *Lancet* II: 572-575.

McCormack CC, Hobson AH, Doyle S, Jackson J, Kilty C & Whitehead AS, 1996. Generation of soluble recombinant human acute phase serum amyloid A2 (A-SAA2) protein and its use in development of a A-SAA specific ELISA. *J Immunol Methods* 198: 101-110.

McGuire TC & Henson JB, 1969. The detection of intravascular haemolysis in the horse. *Br Vet J* 125: v-vi.

Meek RL & Benditt EP, 1986. Amyloid A gene family expression in different mouse tissues. *J Exp Med* 164: 2006-2017.

Meek RL & Benditt EP, 1989. Rat tissues express serum amyloid A protein-related mRNAs. *Prc Natl Acad Sci USA* 86:1890-1894.

Meek RL, Eriksen N & Benditt EP, 1989. Serum amyloid A in the mouse. Sites of uptake and mRNA expression. *Am J Pathol* 135: 411-419.

Meek RL, Urieli-Shoval S & Benditt EP, 1994. Expression of apolipoprotein serum amyloid A mRNA in human atherosclerotic lesions and cultured vascular cells: Implications for serum amyloid A function. *Proc Natl Acad Sci USA* 91: 3186-3190.

Millis DL, Hauptman JG & Richter M, 1992. Preoperative and postoperative hemostatic profiles of dogs undergoing ovariohysterectomy. *Cornell Vet* 82: 465-470.

Milne EM, Doxey DL, Kent JE & Pemberton A, 1991. Acute phase proteins in grass sickness (equine dysautonomia). *Res Vet Sci* 50: 273-278.

Miwata H, Yamada T, Okada M, Kudo T, Kimura H & Morishima T, 1993. Serum amyloid A protein in acute viral infection. *Arch Dis Child* 68: 210-214.

Morimatsu M, Watanabe A, Yoshimatsu K, Fujinaga T, Okubo M & Naiki M, 1991. Elevation of bovine serum C-reactive protein and serum amyloid P component levels by lactation. *J Dairy Res* 58: 257-261.

Morris EA, McDonald BS, Webb AC & Rosenwasser LJ, 1990. Identification of interleukin-1 in equine osteoarthritic joint effusions. *Am J Vet Res* 51: 59-64.

Müller T, Schindler S, Sprenger H, Steinmetz A, Hocke G, Ebel H, Gemsa D & Lange H, 1992. Prospective analysis of 10 different parameters of acute renal allograft rejection. *Transplant Proc* 24: 2731-2734.

Müller TF, Vogl M, Neumann MC, Lange H, Grimm M & Müller MM, 1998. Noninvasive monitoring using serum amyloid A and serum neopterin in cardiac transplantation. *Clin Chim Acta* 276: 63-74.

Nakayama T, Sonoda S, Urano T, Yamada T & Okada M, 1993. Monitoring both serum amyloid protein A and C-reactive protein as inflammatory markers in infectious diseases. *Clin Chem* 39: 293-297.

Nosslin BF & Nyman M, 1958. Haptoglobin determination in diagnosis in haemolytic diseases. *Lancet* 1: 1000-1001.

Nunokawa Y, Fujinaga T, Taira T, Okumura M, Yamashita K, Tsunoda N & Hagio M, 1993. Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. *J Vet Med Sci* 55: 1011-1016.

Odink J, Smeets JFM, Visser IJR, Sandman H & Snijders JMA, 1990. Haematological and clinicochemical profiles of healthy swine and swine with inflammatory processes. *J Anim Sci* 68: 163-170.

Okumura M, Tagami M & Fujinaga T, 1998. Measurement of serum and synovial fluid keratan sulphate and antibody to collagen type II in equine osteoarthritis. *J Vet Med A* 45: 513-516.

Olsson N, Siegbahn A & Nilsson G, 1999. Serum amyloid A induces chemotaxis of human mast cells by activating a pertussis toxin-sensitive signal transduction pathway. *Biochem Biophys Res Commun* 254: 143-146.

Osmand AP, Friedenson B, Gewurz H, Painter RH, Hofmann T & Shelton E, 1977. Characterization of C-reactive protein and the complement subcomponent C1t as homologous proteins displaying cyclic pentameric symmetry (pentraxins). *Proc Natl Acad Sci USA* 74: 739-743.

Paradis MR, 1994. Update on neonatal septicemia. *Vet Clin North Am: Equine Pract* 10: 109-135.

Patel H, Fellowes R, Coade S & Woo P, 1998. Human serum amyloid A has cytokine-like properties. *Scand J Immunol* 48: 410-418.

Pellegrini A, Zweifel H-R & Von Fellenberg R, 1983. Isolation and characterization of horse α_2-macroglobulin protease inhibitor. *Int J Biochem* 15: 1003-1011.

Pepys MB, Baltz ML, Tennent GA, Kent J, Ousley J & Rossdale PD, 1989. Serum amyloid A protein (SAA) in horses: objective measurement of the acute phase response. *Equine Vet J* 21: 106-109.

Pfeffer A & Rogers KM, 1989. Acute phase response of sheep: changes in the concentrations of ceruloplasmin, fibrinogen, haptoglobin and the major blood cell types associated with pulmonary damage. *Res Vet Sci* 46: 118-124.

Poole S, Walker D, Gaines Das RE, Gallimore JR & Pepys MB, 1998. The first international standard for serum amyloid A protein (SAA). Evaluation in an international collaborative study. *J Immunol Methods* 214: 1-10.

Preciado-Patt L, Levartowsky D, Prass M, Hershkoviz, Lider O & Fridkin M, 1994. Inhibition of cell adhesion to glycoproteins of the extracellular matrix by peptides corresponding to serum amyloid A. Toward understanding of the physiological role of an enigmatic protein. *Eur J Biochem* 223: 35-42.

Ray A & Ray BK, 1997. Serum amyloid A gene expression level in liver in response to different inflammatory agents is dependent upon the nature of activated transcription factors. *DNA Cell Biol* 16: 1-7.

Ray BK & Ray A, 1991. Rabbit serum amyloid A gene: cloning, characterization and sequence analysis. *Biochem Biophys Res Commun* 180: 1258-1264.

Raynes JG & McAdam KPWJ, 1988. Purification of serum amyloid A and other high density apolipoproteins by hydrophobic interaction chromatography. *Anal Biochem* 173: 116-124.

Raynes JG & McAdam KPWJ, 1991. Serum amyloid A isoforms in inflammation. *Scand J Immunol* 33: 657-666.

Righetti PG, 1983. Isoelectric focusing: theory, methodology and applications.Chapter 4: General experimental aspects. In *Laboratory Techniques in Biochemistry and Molecular Biology.* Eds TS Work & RH Burdon, Elsevier Biomedical Press, Amsterdam, The Netherlands, pp 268-313.

Robey FA, Jones KD, Tanaka T & Liu TY, 1984. Binding of C-reactive protein to chromatin and nucleosome core particles. A possible physiological role of C-reactive protein. *J Biol Chem* 259: 7311-7316.

Robey FA & Liu TY, 1981. Limulin: a C-reactive protein from *Limulus polyphemus*. *J Biol Chem* 256: 969-975.

Robinson JA, Allen GK, Green EM, Fales WH, Loch WE & Wilkerson CG, 1993. A prospective study of septicaemia in colostrum-deprived foals. *Equine vet J* 25: 214-219.

Rokita H, Shirahama T, Cohen AS, Meek RL, Benditt EP & Sipe JD, 1987. Differential expression of the amyloid SAA 3 gene in liver and peritoneal macrophages of mice undergoing dissimilar inflammatory episodes. *J Immunol* 139: 3849-3853.

Rosenthal CJ & Franklin EC, 1975. Variation with age and disease of an amyloid A protein-related serum component. *J Clin Invest* 55: 746-753.

Rosenthal CJ & Sullivan LM, 1979. Serum amyloid A to monitor cancer dissemination. *Ann Intern Med* 91: 383-390.

Rossevatn K, Andresen PK, Sletten K Husebekk A, Husby G, Nordstoga K, Johnson KH, Westermark GT & Westermark P, 1992. The complete amino acid sequence of bovine serum amyloid protein A (SAA) and of subspecies of the tissue-deposited amyloid fibril protein A. *Scand J Immunol* 35: 217-224.

Roy RB, Shaw RW & Connell GE, 1969. A simple method for the quantitative determination of serum haptoglobin. *J Lab Clin Med* 74: 698-704.

Rygg M, Husby G & Marhaug G, 1993a. Differential expression of rabbit serum amyloid A genes in response to various inflammatory agents. *Scand J Immunol* 38: 417-422.

Rygg M, Marhaug G, Husby G & Dowton SB, 1991. Rabbit serum amyloid protein A: expression and primary structure deduced from cDNA sequences. *Scand J Immunol* 34: 727-734.

Rygg M, Nordstoga K, Husby G & Marhaug G, 1993b. Expression of serum amyloid A genes in mink during induction of inflammation and amyloidosis. *Biochim Biophys Acta* 1216: 402-408.

Sack GH & Talbot CC Jr, 1989. The human serum amyloid A (SAA)-encoding gene *GSAA1*: nucleotide sequence and possible autocrine-collagenase-inducer function. *Gene* 84: 509-515.

Sack GH & Zink MC, 1992. Serum amyloid A gene transcription in synovial cells during retroviral arthritis. *Am J Pathol* 141: 525-529.

Saïle R, Delpierre C, Puchois P, Hocke G, Cachera C, Gesquiere JC, Steinmetz A, Tartar A & Fruchart JC, 1988. Enzyme-linked immunosorbent assay for serum amyloid A apolipoprotein with use of specific antibodies against synthetic peptides. *Clin Chem* 34: 1767-1771.

Saini PK, Riaz M, Webert DW, Eckersall PD, Young CR, Stanker LH, Chakrabarti E & Judkins JC, 1998. Development of a single enzyme immunoassay for blood haptoglobin concentration in cattle and its application in improving food safety. *Am J Vet Res* 59: 1101-1107.

Satoh M, Fujinaga T, Okumura M & Hagio M, 1995. Sandwich enzyme-linked immunosorbent assay for quantitative measurement of serum amyloid A protein in horses. *Am J Vet Res* 56: 1286-1291.

Schrödl W, Kruger M, Hien TT, Fuldner M & Kunze R, 1995. Das C-reaktive Protein als ein neuer Parameter bei Mastitis. [C-reactive protein as a new parameter of mastitis.] *Tierarztl Prax* 23: 337-341.

Sellar GC, De Beer MC, Lelias JM, Snyder PW, Glickman LT, Felsburg PJ & Whitehead AS, 1991. Dog serum amyloid A protein. Identification of multiple isoforms defined by cDNA and protein analyses. *J Biol Chem* 266: 3505-3510.

Shainkin-Kestenbaum R, Zimlichman S, Lis M, Preciado-Patt, Fridkin M & Berenheim J, 1996. Modulation of prostaglandin I_2 production from bovine aortic endothelial cells by serum amyloid A and its N-terminal tetradecapeptide. *Biomed Pept Proteins Nucleic Acids* 2: 101-106.

Shainkin-Kestenbaum R, Zimlichman S, Winikoff Y, Pras M, Chaimovitz C & Sarov I, 1982. Serum amyloid A (SAA) in viral infection: rubella, measles and subacute sclerosing panencephalitis (SSPE). *Clin exp Immunol* 50: 503-506.

Sipe JD, Colten HR, Goldberger G, Edge MD, Tack BF, Cohen AS & Whitehead, AS, 1985. Human serum amyloid A (SAA): Biosynthesis and postsynthetic processing of preSAA and structural variants defined by complementary DNA. *Biochemistry* 24: 2931-2936.

Sipe JD, Gonnerman WA, Loose LD, Knapschaefer G, Xie W-J & Franzblau C, 1989. Direct binding enzyme-linked immunosorbent assay (ELISA) for serum amyloid A (SAA). *J Immunol Methods* 125: 125-135.

Sipe JD, Ignaczak TF, Pollock PS & Glenner GG, 1976. Amyloid fibril protein AA: purification and properties of the antigenically related serum component as determined by solid phase radioimmunoassay. *J Immunol* 116: 1151-1156.

Skinner JG, Brown RALI & Roberts L, 1991. Bovine haptoglobin response in clinically defined field conditions. *Vet Rec* 128: 147-149.

Skogen B, Børresen AL, Natvig JB, Berg K & Michaelsen TE, 1979. High-density lipoprotein as a carrier for amyloid-related protein SAA in rabbit serum. *Scand J Immunol* 10: 39-45.

Sletten K, Husebekk A & Husby G, 1989. The primary structure of equine serum amyloid A (SAA) protein. *Scand J Immunol* 30: 117-122.

Sletten K, Marhaug G & Husby G, 1983. The covalent structure of amyloid-related serum protein SAA from two patients with inflammatory disease. *Hoppe-Seyler's Z Physiol Chem* 364: 1039-1046.

Smith JE & Cipriano JE, 1987. Inflammation-induced changes in serum iron analytes and ceruloplasmin of Shetland ponies. *Vet Pathol* 24: 354-356.

Smith JW, Colombo JL & McDonald TL, 1992. Comparison of serum amyloid A and C-reactive protein as indicators of lung inflammation in corticosteroid treated and non-corticosteroid treated cystic fibrosis patients. *J Clin Lab Anal* 6: 219-224.

Spector TD, Hart DJ, Nandra D, Doyle DV, Mackillop N, Gallimore JR & Pepys MB, 1997. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. *Arthritis Rheum* 40: 723-727.

Stearman RS, Lowell CA, Peltzman CG & Morrow JF, 1986. The sequence and structure of a new serum amyloid A gene. *Nucleic Acids Res* 14: 797-809.

Steel DM, Sellar GC, Uhlar CM, Simon S, De Beer FC & Whitehead AS, 1993. A constitutively expressed serum amyloid A protein gene (*SAA4*) is closely linked to, and shares structural similarities with, an acute-phase serum amyloid A protein gene (*SAA2*). *Genomics* 16: 447-454.

Sweeney CR, Sweeney RW & Divers TJ, 1987. *Rhodococcus equi* pneumonia in 48 foals: response to antimicrobial therapy. *Vet Microbiol* 14: 329-336.

Syversen PV, Juul J, Marhaug G, Husby G & Sletten K, 1994a: The primary structure of serum amyloid A protein in the sheep: Comparison with serum amyloid A in other species. *Scand J Immunol* 39: 88-94.

Syversen PV, Juul J, Rygg M, Sletten K, Husby G & Marhaug G, 1993a. The primary structure of rabbit serum amyloid A protein isolated from acute phase serum. *Scand J Immunol* 37: 447-451.

Syversen PV, Sæter U, Cunha-Ribeiro L, Ørvim U, Sletten K, Husby G & Sakariassen KS, 1994b. The effect of serum amyloid protein A fragment-SAA_{25-76} on blood platelet aggregation. *Thromb Res* 76: 299-305.

Syversen PV, Sletten K, Marhaug G, Husby G & Lium B, 1993b. The revised amino acid sequence of serum amyloid A (SAA) in mink. *Scand J Immunol* 37: 696-697.

Taira T, Fujinaga T, Tamura K, Izumi M, Itoh H, Tsunoda N, Yamashita K, Okumura M & Mizuno S, 1992. Isolation and characterisation of α_1-acid glycoprotein from horses, and its evaluation as an acute-phase reactive protein in horses. *Am J Vet Res* 53: 961-965.

Tatum F, Alam J, Smith A & Morgan WT, 1990. Molecular cloning, nucleotide sequence heterozygosity and regulation of rabbit serum amyloid A cDNA. *Nucleic Acids Res* 18: 7447.

Tillett WS & Francis T Jr, 1930. Serological reactions in pneumonia with a non-protein somatic fraction of *Pneumococcus*. *J Exp Med* 52: 561-571.

Todhunter RJ, Fubini SL, Freeman KP & Lust G, 1997. Concentrations of keratan sulfate in plasma and synovial fluid from clinically normal horses and horses with joint disease. *J Am Vet Med Assoc* 210: 369-374.

Toussaint MJM, Van Ederen AM & Gruys E, 1995. Implication of clinical pathology in assessment of animal health and in animal production and meat inspection. *Comp Haematology Int* 5: 149-157.

Turnell W, Sarra R, Glover ID, Baum JO, Caspi D, Baltz ML & Pepys MB, 1986. Secondary structure prediction of human SAA_1 presumptive identification of calcium and lipid binding sites. *Mol Biol Med* 3: 387-407.

Urieli-Shoval S, Cohen P, Eisenberg S & Matzner Y, 1998. Widespread expression of serum amyloid A in histologically normal human tissues: predominant localisation to the epithelium. *J Histochem Cytochem* 46: 1377-1384.

Urieli-Shoval S, Meek RL, Hanson RH Eriksen N & Benditt EP, 1994. Human serum amyloid A genes are expressed in monocyte / macrophage cell lines. *Am J Pathol* 145: 650-660.

Van der Kolk H, 1991. Some aspects of the bovine pituitary-adrenocortical axis with special reference to persistent udder oedema. *Thesis*, University of Utrecht, Utrecht, The Netherlands, pp 73-82.

Van Leeuwen MA & Van Rijswijk, MH, 1994. Acute phase proteins in the monitoring of inflammatory disorders. *Bailliere's Clinical Rheumatology: Reactive Amyloidosis and the Acute Phase Response* 8: 531-552.

Van Rijswijk MH, 1981. Amyloidosis. *Thesis,* University of Groningen, The Netherlands.

Von den Velden R, 1914. Die Blutgerinnung nach parenteraler Zuführ von Eiweisskörpern. *Dtsch Arch J Klin Med* 41: 298-326.

Webb CF, Tucker PW & Dowton SB, 1989. Expression and sequence analyses of serum amyloid A in the Syrian hamster. *Biochemistry* 28: 4785-4790.

Whicher JT, Banks RE, Thompson D & Evans SW, 1993. The measurement of acute phase proteins as disease markers. In *Acute Phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications.* Eds: A Mackiewicz, I Kushner & H Baumann, CRC Press, Boca Raton, 633-650.

Whicher JT, Chambers RE, Higginson J, Nashef L & Higgins PG, 1985. Acute phase response of serum amyloid A protein and C reactive protein to the common cold and influenza. *J Clin Pathol* 38: 312-316.

Whitehead AS, De Beer MC, Steel DM, Rits M, Lelias JM, Lane WS & De Beer FC, 1992. Identification of novel members of the serum amyloid A protein superfamily as constitutive apoproteins of high density lipoprotein. *J Biol Chem* 267: 3862-3867.

Whitehead AS, Zahedi K, Rits M, Mortensen RF & Lelias JM, 1990. Mouse C-reactive protein. Generation of cDNA clones, structural analysis, and induction of mRNA during inflammation. *Biochem J* 266: 283-290.

Wilke G, Rath W, Schutz E, Armstrong VW & Kuhn W, 1992. Haptoglobin as a sensitive marker of hemolysis in HELLP-syndrome. *Int J Gynaecol Obstet* 39: 29-34.

Wilkins J, Gallimore R, Tennent GA, Hawkins PN, Limburg PC, Van Rijswijk MH, Moore EG & Pepys MB, 1994. Rapid automated enzyme immunoassay of serum amyloid A. *Clin Chem* 40: 1284-1290.

Willett K & Blackmore DJ, 1979. Haptoglobin in the serum of thoroughbreds in training. *Res Vet Sci* 26: 308-314.

Wood DD, Gammon M & Staruch MJ, 1982. An ELISA assay for murine amyloid A and serum amyloid A utilizing monoclonal antibodies. *J Immunol Methods* 55: 19-26.

Yamada T, Kakihara T, Kamishima T, Fukuda T & Kawai T, 1996. Both acute phase and constitutive serum amyloid A are present in atherosclerotic lesions. *Pathol Int* 46: 797-800.

Yamada T, Nomata Y, Sugita O & Okada M, 1993. A rapid method for measuring serum amyloid A protein by latex agglutination nephelometric immunoassay. *Ann Clin Biochem* 30: 72-76.

Yamada T, Uchiyama K, Yakata M & Gejyo F, 1989. Sandwich enzyme immunoassay for serum amyloid A protein (SAA). *Clin Chim Acta* 179: 169-176.

Yamamoto K & Migita S, 1985. Complete primary structures of two major murine serum amyloid A proteins deduced from cDNA sequences. *Proc Natl Acad Sci USA* 82: 2915-2919.

Yamamoto S, Miyaji S, Ashida Y, Otabe K, Momotani E & Rikihisa Y, 1994. Preparation of anti-canine serum amyloid A (SAA) serum and purification of SAA from canine high-density lipoprotein. *Vet Immunol Immunopathol* 41: 41-53.

Yamashita K, Fujinaga T, Miyamoto T, Hagio M, Izumisawa Y & Kotani T, 1994. Canine acute phase response: relationship between serum cytokine activity and acute phase protein in dogs. *J Vet Med Sci* 56: 487-492.

Yamashita K, Fujinaga T, Okumura M, Takiguchi M, Tsunoda N & Mizuno S, 1991. Serum C-reactive protein (CRP) in horses: The effect of aging, sex, delivery and inflammations on its concentration. *J Vet Med Sci* 53: 1019-1024.

Zimlichman S, Danon A, Nathan I, Mozes G & Shainkin-Kestenbaum R, 1990. Serum amyloid A, an acute phase protein, inhibits platelet activation. *J Lab Clin Med* 116: 180-186.

Zuckerman SH & Surprenant YM, 1986. Simplified microELISA for the quantitation of murine serum amyloid A protein. *J Immunol Methods* 92: 37-43.

Acknowledgements

The work presented in this thesis was carried out at the Department of Clinical Chemistry, SLU, Uppsala in co-operation with the Department of Paediatrics, Institute of Clinical Medicine, University of Tromsø, Norway.
Substantial support has also been obtained from the Department of Large Animal Clinical Sciences, SLU, Uppsala; Department of Clinical Veterinary Sciences, Helsinki University, Finland; Bayer AB, Gothenburg, Sweden and Department of Biochemistry, University of Oslo, Norway.
Financial support has been provided from SLU, Michael Forsgren's Foundation, and from the Swedish Horse Race Totalisator Board.

I would like to take the opportunity to thank:

Mats Forsberg, my scientific supervisor, for guidance and good team work, for being straightforward and visionary, for being rapid instead of in a hurry, for encouraging discussions on scientific and other subjects and for putting up with my somewhat slower pace.

Gudmund Marhaug, my associate scientific supervisor and a very important person in this work, for generously inviting me to Tromsø (sometime in the beginning of time) and for unprestigiously sharing his knowledge on SAA through the years, for encouragement and feedback and for being an inspiring, relaxed and pleasant person to be with.

Lars-Erik Edqvist, my former scientific supervisor, for giving me the opportunity to work at the department and for being far-sighted, patient and cool in the beginning of this project.

Lennart Thunberg, my former associate scientific supervisor, for being a source of knowledge in matters of protein purification and chromatography, and for nice chats about the high-lights of family life.

Bernt Jones, Head of Department of Clinical Chemistry, for sharing his comprehensive knowledge whenever asked.

Cathrine Foyn Bruun, my co-author and friend, for leading me through the mysterious labyrinths of 2D protocols and for, together with Marite, introducing me to several north-Norwegian habits and delights.

Marite Rygg, laboratory mate and friend, for providing multiple-star service and pleasant company during my stays in Tromsø, and for introducing me to the passion and temper in north-Norwegian soccer.

Knut Sletten, co-author, for managing to find isoforms of SAA in the small "scraps of paper" that I sent him.

Karin Burvall, co-author, for handling the SAA assay with professional hands through the years, and for being a most pleasant travel mate (and for having such a good sense of direction!).

Riitta-Mari Tulamo and **Mikko Suominen,** co-authors, for "letting me in" to their experiment and for giving me access to their data (and to Riitta-Mari for encouraging and cheerful greetings!).

Björn Sandgren, co-author, for patiently consulting his old files to find answers to my numerous questions and to Björn, **Eva Skiöldebrand, Berndt Klingeborn** for letting me use their material and for good advice.

Ulrika Grönlund, co-author, for being dedicated and stubborn in the lab, for good co-operation and lots of good chats (and for remembering coffee-milk more often than I did).

Susanne Demmers, co-author, for being my equine-clinical-practice-common-sense consultant and for all work with "the foal samples" and for being relaxed and nice to be around.

Lena Hörnsten, for teaching me the fundamentals of basic laboratory techniques in the very beginning.

Ove Wattle, for sharing my interest in 2D and for being better at it than I am.

Åsa Karlsson, for help with the SAA assay in the early beginning of things.

Berit Ek and **Kerstin Gävner**, for interested and skilful development and handling of haptoglobin measurements and electrophoresis, respectively.

Henrik Holst, for once in a while having assured me that the Department of Clinical Chemistry was the right place to work and for always providing time and atmosphere for a nice chat or a serious discussion in his corner room when still at the department and for reading my thesis in the busiest of times. And most of all, Henrik and **Bodil Ström Holst,** for being very good friends.

Inger Lilliehöök, for being a good friend and for being a guide to common sense in all kinds of matters.

Håkan Andersson, for being an outstanding discussion partner on just any subject.

Permanent and temporary staff at the Department, not mentioned above, for making work and breaks enjoyable.

Stig, my father, for constant support in various ways and for his confidence in my ability.

Therese, my sister, for keeping calling me although I am lousy at calling her and for always seeing things from the bright side.

Adam and **David,** for choosing me as their mother, and for being a constant source of knowledge, life and good ideas.

Fredrik, for being my partner in the project of love and life, for being "up" when I´m "down" and for being an inspiration in doing things your way.

In memoriam

Juhani Hirvonen, my co-author and - although we only met a few times – friend, died in the beginning of August when he was busy finishing his thesis, but most importantly, when he was busy living. Juhani was *alive* in the very best sense, and to know him was a pleasure; he was open, unselfish and humorous. I am greatly indebted to Juhani for his contributions to my thesis work and I miss him.

Acta Universitatis Agriculturae Sueciae
presents doctoral theses from the faculty of agriculture, landscape architecture and horticulture, the faculty of forestry and the faculty of veterinary medicine at the Swedish University of Agricultural Sciences. Each faculty has its own subseries, Agraria, Silvestria and Veterinaria, respectively, with separate numbering.

ISSN 1401-6257
ISBN 91-576-5449-2