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## **PREFACE**

This publication was intended to be the academic dissertation of DVM Juhani Hirvonen. The dissertation was planned to consist of five articles, which have already been published in refereed veterinary journals. Juhani Hirvonen started his thesis work at the College of Veterinary Medicine, now faculty of the University of Helsinki, in 1994, under supervision of professors Satu Pyörälä and Markus Sandholm. Juhani was very interested in his topic, and worked with enthusiasm and joy. During the years since 1994 he was not able to devote all his time for research but worked also as practicing veterinarian in Nilsinä municipality. In summer 1999 Juhani finished the manuscript, and it was ready to be submitted to the referees which would have been nominated by the faculty. Professor Markus Sandholm, one of the supervisors of this work, died in July 1999 and did not have the chance to see the dissertation at its final stage.

The public defence of Juhani's dissertation had been planned to take place in fall 1999, after the manuscript would have been accepted by the referees. This never happened, because Juhani Hirvonen died due of acute brain haemorrhage on the 8<sup>th</sup> September 1999. He collapsed when participating a foot ball match with friends. Juhani Hirvonen was a talented veterinarian and researcher, and a wonderful personality. His work on bovine acute phase response is an important scientific input to the field of acute phase research in animals, and also of clinical relevance to veterinary practice. As the supervisor of Juhani I have finalised this book. It was also reviewed by DVM, Ph.D. Satu Sankari, to whom Juhani sent it in July 1999, and later by DVM, Ph.D. Liisa Kaartinen. All of us made very few comments and corrections in the text. The help of DVM Heli Lindeberg, Juhani's wife was indispensable in the practical questions with the manuscript.

Warmest thanks to professor Hannu Saloniemi, professor Riitta-Mari Tulamo, DVM, Ph.D. Satu Sankari, DVM, Ph.D. Liisa Kaartinen, chief librarian Teodora Oker-Blom and librarian Raisa Iivonen for their kind support and help in getting Juhani's work published. Ph.D. Jonathan Robinson is thanked for revising the English text of the manuscript. The publishing costs of this book were covered by the grant kindly provided by the Finnish Veterinary Science Foundation.

To the memory of Juhani.

Helsinki, 26 November, 1999

Satu Pyörälä

## ORIGINAL ARTICLES

This thesis is based on the following original articles which are referred to in the text by their Roman numerals I - V:

- I. Hirvonen J, Pyörälä S, Jousimies-Somer H. 1996. Acute phase response in heifers with experimentally induced mastitis. *J Dairy Res* 63:351-360.
- II. Hirvonen J, Pyörälä S. 1997. Acute-phase response in dairy cows with surgically-treated abdominal disorders. *Vet Journal* 155: 53-61.
- III. Hirvonen J, Hietakorpi S, Saloniemi H. 1997. Acute phase response in emergency slaughtered dairy cows. *Meat Science* 46: 249-257.
- IV. Hirvonen J, Eklund K, Teppo AM, Huszenicza G, Kulcsar M, Saloniemi H, Pyörälä S. 1999. Acute phase response in dairy cows with experimentally induced *Escherichia coli* mastitis. *Acta Vet Scand* 40: 35-46.
- V. Hirvonen J, Huszenicza G, Kulcsar M, Pyörälä S. 1999. Acute phase response in dairy cows with acute postpartum metritis. *Theriogenology* 51: 1071-1083.

## ABBREVIATIONS

$\alpha_1$ -AG	Alpha <sub>1</sub> -acid glycoprotein
$\alpha_1$ -PI	Alpha <sub>1</sub> -proteinase inhibitor
$\alpha_1$ -AT	Alpha <sub>1</sub> -antitrypsin
APP	Acute phase protein(s)
APR	Acute phase response
ASG	Acid soluble glycoproteins
AUC	Area under curve
ASAT	Aspartate aminotransferase
BAPNA	<i>N</i> -benzoylarginine- <i>p</i> -nitroanilide
BRD	Bovine respiratory disease
BUN	Blood urea nitrogen
Cp	Ceruloplasmin
CFU	Colony forming unit
CK	Creatine kinase
CRP	C-reactive protein
HbCN	Cyanmethaemoglobin
ELISA	Enzyme-linked immunosorbent assay
Fb	Fibrinogen
GFR	Glomerular filtration rate
Hb	Haemoglobin
HbBC	Haemoglobin-binding capacity
Hp	Haptoglobin
HDL	High-density lipoprotein
HPLC	High performance liquid chromatography
kD	Kilodalton
MIC	Minimum inhibitory concentration
NAGase	<i>N</i> -acetul- $\beta$ -D-glucosaminidase
NO <sub>3</sub>	Nitrate
NO	Nitric oxide
NO <sub>2</sub>	Nitrite
PCV	Packed cell volume
RBC	Red blood cell
RP	Retained placenta
ROC	Receiver operating characteristics
SAA	Serum amyloid-A
SAP	Serum amyloid-P
SCC	Somatic cell count
TNF $\alpha$	Tumor necrosis factor -alpha
TIC	Trypsin-inhibitory capacity

## SUMMARY

The term *acute phase response* (APR) refers to the inflammatory response of the host occurring shortly after any tissue injury. The purpose of the APR is to prevent further injury of an organ, to limit the growth of the infective organism, to remove harmful molecules, and to activate the repair processes to return the organ to normal function. APR is characterized by the systemic inflammatory signs, such as fever, inappetite and depression, which are a reflection of multiple endocrinological, haematological, immunological, metabolic, and neurological changes in the diseased animal. Most frequently, the term APR is used to refer to the changes in concentrations of a number of liver-derived plasma proteins that are associated with this response. Those proteins which markedly increase their plasma concentration are called *acute phase proteins* (APP). The APR is part of the non-specific immune response, being followed by the specific immune response.

Despite the highly conserved nature of the APR, the plasma APP profiles between different animal species differ. For most bovine APP, their characteristics in different pathological conditions have not yet been described in detail. The major plasma APPs in the cow include haptoglobin (Hp) and serum amyloid-A (SAA). Other bovine APP are either weakly or moderately responding plasma proteins, such as  $\alpha_1$ -acid glycoprotein,  $\alpha_1$ -proteinase inhibitor and fibrinogen. The aim of this study was to investigate the characteristics of bovine APR and evaluate the diagnostic and prognostic capacity of bovine APP in various clinical diseases of dairy cattle. The bovine APR was examined horizontally, including both sequential clinical examinations and plasma/serum APP determinations. The material consisted of two experimental mastitis trials with dairy cattle and clinical material including dairy cows with surgically treated abdominal disorders, acute postpartum metritis, and emergency slaughtered dairy cows.

According to the APP profiles, APR played a role in all clinical conditions involved in this study. The intensity of APR varied between different disorders, being highest in bacterial diseases, particularly in purulent infections. Typical diseases with high APR were mastitis, arthritis and traumatic reticuloperitonitis. Acute postpartum metritis produced a variable APR. Abdominal surgery induced only a minor to moderate APR without a marked effect on the host. Emergency slaughtered dairy cows usually had a moderately high APR. The susceptibility of the individual heifers and cows to experimental mastitis varied strongly. According to clinical signs and bacterial recovery, the experimental animals were clearly different to those with a mild and short-lived infection, and those with a more severe and persistent one. The severity of disease was also reflected in the APP patterns.

APP proved to be sensitive markers for various inflammatory conditions of dairy cows, the sensitivity being better than that of clinical examination and conventional haematology. As already known, the determined APP fall into different categories according to their response patterns. Blood  $\alpha_1$ -AG,  $\alpha_1$ -PI and Fb produced a mild to moderate relative response, and the response patterns were rather similar between individual animals, whereas blood Hp and SAA have a high relative response, with more variability between individuals. Hp proved to be a good diagnostic and prognostic marker for purulent and



more chronic infections, whereas SAA was an accurate marker in more acutely diseased animals. One advantage of Hp and SAA over the clinical examination is the better quantification of disease severity than obtained by clinical examination alone.

In conclusion, APP were of value in predicting the outcome of disease. For predicting bacterial recovery, the accuracy was best in purulent bacterial diseases. In the case of an acute uterine infection, high APP levels may reflect a severe infection which can reduce fertility. APP can also play a role in meat inspection. Indications for their use can be discrimination between healthy and diseased animals, and quantification of the inflammatory status of slaughter cattle.

## **INTRODUCTION**

When a veterinary surgeon examines a diseased animal during a farm call, the animal is often suffering from acute phase response (APR). APR is a physiological condition taking place at the very beginning of the inflammatory process, being independent of the origin of inflammation. In case of acutely diseased animals, the diagnosis and choice of treatment are usually based on observations made from both local and systemic clinical signs which, in fact, all are consequences of the APR. Therefore, it is important for a veterinary practitioner to understand the nature of this phenomenon to be able to interpret correctly the results of the physical examination.

### **Acute phase response (APR)**

The term *acute phase response* refers to the inflammatory response of the host occurring shortly after any tissue injury (Kushner 1982, Dinarello 1984, Baumann & Gauldie 1994, Raynes 1994, Pannen & Robotham 1995, Koj 1996). The APR is non-specific by nature: the origin of the injury can be infective, immunologic, neoplastic, traumatic, parasitic or other (Kushner & Mackiewicz, 1987, Stadnyk & Gauldie 1991). In its narrow sense, the term APR refers to the changes in concentrations of a large number of plasma proteins that are associated with the host response. These changes are predominantly the result of alterations in the pattern of protein synthesis in the liver (Pannen & Robotham 1995). The purpose of the APR is to prevent further injury of an organ, to isolate and destroy the infective organism, to remove the harmful molecules and debris, and to activate the repair processes that are necessary to return the organ to its normal function (Dinarello 1984, Baumann & Gauldie, 1994). From a teleological point of view, APR is a primitive event that helps to permit survival and maintain physiologic homeostasis during the period following injury (Dinarello 1984). The APR is part of the non-specific immune response, and its various components are relatively consistent despite the large variety of conditions that induce it. The APR is later followed by the specific immune response which in contrast is selective.

### *Initiation of APR*

The initiation of the APR takes place at the site of injury. The inflammatory cascade is usually started by mononuclear cells, i.e. tissue macrophages or blood monocyte cells. They are able to release a broad spectrum of inflammatory mediators, such as cytokines, lipid mediators, vasoactive amines, products of the complement and coagulation cascades, proteases, reactive oxygen species, and nitric oxide (Olson *et al.* 1995; Monshouwer *et al.* 1996). The inflammatory mediators trigger both the local and systemic inflammatory reactions. The local reactions include increase in capillary permeability, and infiltration of leucocytes to the area of inflammation. The increased capillary permeability allows the transport of different molecules between circulation and the area of tissue injury. These molecules consist of many plasma proteins, such as proteinase inhibitors, transport proteins, and other binding proteins. Also many ions are transferred to the area, e.g. Na<sup>+</sup> and Cl<sup>-</sup>. The migration of leucocytes into the inflammatory site is regulated by their adhesion to the endothelium. Leucocytes and capillary endothelial cells express adhesion surface receptors in response to inflammatory mediators. The adherence of leucocytes to endothelium is followed by diapedesis of the leucocytes and their migration to the inflammatory focus under guidance of different chemotactic factors. Phagocytic cells, neutrophilic granulocytes and macrophages, play a key role in eliminating foreign antigens. Their function is based on phagocytosis, lysosomal hydrolases, and oxygen radicals. Two oxygen radicals, superoxide anion (O<sub>2</sub><sup>-</sup>) and nitric oxide (NO<sup>-</sup>), can also be converted to peroxylnitrite (ONOO<sup>-</sup>) which has recently been implicated as a major cytotoxic agent (Paape & Capuco 1997).

Table 1. Initiation of the APR

Tissue trauma	Injury Infection Necrosis Neoplasm Immunological disorder
Elaboration of alarm molecules	Proteases Free radicals Lipid derivatives Vasoactive amines Products of complement and coagulation cascades
Activation of responsive cells	Macrophages Fibroblasts Endothelial cells Other cells
Synthesis and release of pro-inflammatory cytokines	Interleukin-1 Interleukin-6 Tumor necrosis factor- $\alpha$ Interferon- $\gamma$
Initiation of systemic APR and APP production	Described in Table 2.

Cytokines are multipotent polypeptides produced by various cell types. Their synthesis is initiated by the above mentioned inflammatory mediators, which induce the cascade of signal transduction, transcription of cytokine genes, translation into cytokine polypeptide, and its processing and secretion (Koj 1996).

Pro-inflammatory cytokines, such as tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon- $\gamma$  (IFN- $\gamma$ ) appear to be essential for initiating the systemic inflammatory response (Kushner 1993, Baumann & Gauldie 1994, Koj 1996, Murtaugh *et al.* 1996). At the local reaction site, these cytokines activate stromal cells, such as fibroblasts and endothelial cells, to initiate the secondary release of cytokines (Baumann & Gauldie 1994). This secondary wave and the appearance of these early cytokines in the circulation is responsible for the start of the systemic inflammatory response.

### *Continuation of APR*

The APR is clinically characterized by the systemic inflammatory signs, fever, inappetite, and depression. These symptoms reflect multiple changes in the homeostatic control of the diseased animal, which are described in general in the following paragraphs.

### *Endocrinological changes*

The APR includes many endocrinological changes. One of them is the stimulation of adrenocorticotrophic hormone (ACTH) production in the hypothalamus and the subsequent production of cortisol in the adrenal cortex (Paape *et al.* 1974; Boosman *et al.* 1990). Also serum concentration of many other hormones increase: these include adrenal catecholamines, glucagon, insulin, growth hormone, aldosterone, vasopressin, and prolactin (Kushner 1982, Dinarello 1984, Mandrup-Poulsen *et al.* 1995). Some other hormones decrease in concentration during the acute phase; these are renin, thyroxine, as well as gonadal steroids (Mandrup-Poulsen *et al.* 1995). It must be noted that the reports about the hormonal patterns during APR are partly controversial, and different results have been obtained from different animal species. Also the background of the endocrinological changes is poorly understood, but one aim of these changes could be stimulation of the energy metabolism of the host.

### *Metabolic changes*

The main metabolic changes during APR are increased protein catabolism and gluconeogenesis. The muscle proteins are catabolized to amino acids that are required for synthesis of new proteins at a time when food intake is reduced. Amino acids are required for the synthesis of hepatic acute phase proteins, immunoglobulins, and collagen for tissue repair, and the proliferation of lymphocyte and fibroblast cells. Amino acids are used also for gluconeogenesis and energy. Despite the anabolic processes, the catabolism of muscle proteins results in negative nitrogen balance and weight loss of the diseased animal. Some central organs are preferentially preserved from this catabolism, i.e. kidney, liver and lung. The reason for this is not clear but these tissues are major components of the reticuloendothelial system which frequently increases its activity during APR (Jennings & Elia 1996). Unfortunately, there are only limited data on these events available from

domestic animal species. One of the most important metabolic changes is the strongly increased synthesis of a group of plasma proteins, namely acute phase proteins (APP), in the liver (Kushner 1982, Eckersall & Conner 1988, Pannen & Robotham 1995, Baumann & Gauldie 1994, Gruys *et al.* 1994, Raynes 1994). These proteins are discussed in detail later.

### *Haematological changes*

Alterations in serum cation concentrations occur during the APR (Kushner 1982). Zinc and iron concentrations decline substantially, whereas plasma copper concentration may increase (Lohuis *et al.*, 1988 and 1988b, Hayes 1994). These ion changes reflect changes in cation binding of plasma proteins, and more importantly, alterations in cellular uptake mechanisms. In cattle, initial leukopenia and a left shift is one of the major findings during APR. Leukopenia is derived from stress-induced decrease of lymphocytes and emigration of neutrophils into the inflammatory focus. As the reserve of mature neutrophils becomes depleted, immature neutrophils enter the circulation resulting in a degenerative left shift (Kidd 1991, Jain 1993, Cole *et al.* 1997). Within several hours of the initial drop in mature neutrophils, intramedullary granulopoiesis becomes stimulated, sometimes resulting in a rebound neutrophilia within 1-2 days after the onset of the acute inflammatory disease; this neutrophilia may be more pronounced in young calves because of their larger reserve neutrophil pools. In older cattle, bone marrow requires 4-5 days to replenish immature neutrophils in circulation (Cole *et al.* 1997). Bovine haematological changes during APR include activation of haemostatic mechanisms, such as platelet function and clotting cascade (Deldar *et al.* 1984, Welles *et al.* 1993, Cheryk *et al.* 1998). The complementary pathway is also activated during APR (Koj 1996).

### *Neurological changes*

APR initiates many neurological changes in the host. Somnolence during the acute phase is due to depression of central nervous system. The inflamed area is usually painful: pain is mediated through vasoactive amines, such as bradykinin (Baumann & Gauldie, 1994).

### *Immunomodulation*

APR also has many immunosuppressive properties such as lymphocyte hyporeactivity, and decrease of neutrophil bactericidal and macrophage phagocytical activity (Kohler & Prokop, 1978; Kushner 1982).

*Table 2. Characteristics of the systemic APR.*

Clinical signs	Fever Inappetite Somnolence
Endocrinological changes	ACTH and cortisol↑ Adrenal catecholamines↑ Glucagon and insulin↑ Growth hormone↑ Thyroxin↓ Gonadal steroids↓
Metabolic changes	Protein catabolism↑ Gluconeogenesis ↑ Hepatic production of APP↑ Reticuloendothelial system↑↓
Hematological changes	Zinc and iron↓, copper↑ Leukopenia and left shift Platelet function↑
Immunological changes	Lymphocyte reactivity↓ Neutrophil bacterial killing↓ Macrophage phagocytosis↓
Neurological changes	Depression of CNS Pain (vasoactive amines ↑)

Cytokines play a major role as immunomodulators (Koj 1996), and also many liver-derived APP are known to have such properties (James 1990; Motoi *et al.*, 1993, Sato *et al.*, 1995). The APR is followed by the specific immune response, including antigen processing and presentation, T-cell proliferation, and B-cell originated antibody production. It is evident that these two mechanisms interact strongly.

### *Downregulation of APR*

The downregulation of APR involves many inflammatory mediators, such as glucocorticoids, cytokines including interleukin-4 (IL-4) and IL-10, and receptor antagonists for certain pro-inflammatory cytokines (Besedovsky *et al.* 1986, Baumann & Gauldie 1994, Koj 1995). The APR subsides over 1-2 days, and the host returns to normal function. The APR can also be prolonged if acute inflammation develops to chronic (Baumann & Gauldie 1994). Several physiological and pathophysiological phenomena affect the manifestation of APR. For example, malnourishment can attenuate APR (Jennings & Elia 1996). We previously found that repeated challenge of the bovine mammary gland with *Escherichia coli* was followed by a suppressed APR (Salonen *et al.* 1996).

### *Pharmacological aspects of APR*

APR affects drug kinetics by many mechanisms. Nutrient and water intake usually decrease during APR. Gastrointestinal function is also altered: forestomach hypomotility and decreased gastric emptying rate affect drug absorption (van Miert 1987). APR also affects drug distribution and metabolism in liver and kidneys (Davis 1986, van Miert 1995). APR affects protein binding of several drugs. The most important protein in this respect is probably  $\alpha_1$ -AG, an APP which binds basic drugs, like trimethoprim and erythromycin (Kremer *et al.* 1988, Tagawa *et al.* 1994, Son *et al.* 1996). Many enzymes that metabolize drugs are down-regulated during the acute phase (van Miert 1995).

### **Acute phase proteins (APP)**

#### *Determination of APP*

APR alters the synthesis and release of many proteins synthesized by the liver; some of which decrease and others increase. Those proteins that decrease are termed negative acute phase proteins; to this group belong albumin and many other binding proteins, e.g. transferrin and retinol binding protein (Jain 1993, Hayes 1994, Gruys *et al.* 1994). Those proteins that increase over 25% in concentration are termed positive acute phase proteins, or simply acute phase proteins (Kushner 1982). Here we refer to this group of proteins when using the term APP. Some APP are secreted into circulation continuously, being



referred as constitutive APP; others exist in plasma only during APR and are referred to as inflammation-induced APP or APR-induced APP.

### *Production of plasma APP*

The synthesis and release of plasma APP from liver is regulated by inflammatory mediators. These mediators fall into four major categories: interleukin-6-type cytokines, interleukin-1-type cytokines, glucocorticoids, and growth factors. Cytokines mainly stimulate the APP gene-expression, while glucocorticoids and growth factors function more as modulators of cytokine action (Baumann & Gauldie 1994). Interleukin-6 (IL-6) has been recognized as the principal regulator of most APP genes. The APP produced are termed type-2 APP; in most species these include fibrinogen (Fb), haptoglobin (Hp) and at least one of the major antiproteases, like  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ -PI). The group of APP genes regulated by interleukin-1-type cytokines (IL-1a, IL-1b, TNF $\alpha$ ) is clearly different from that regulated by IL-6-type cytokines. The APP produced are called type-1 APP, and they include e.g. alpha<sub>1</sub>-acid glycoprotein ( $\alpha_1$ -AG), serum amyloid-A (SAA), and C-reactive protein (CRP), depending on the species (Baumann & Gauldie 1994, Pannen & Robotham 1995, Nakagawa-Tosa *et al.* 1995). The above mentioned classification is not complete: bovine Hp is stimulated by IL-6 and TNF, but not by IL-1 (Nakagawa-Tosa *et al.* 1995). Binding of the inflammatory mediators to their respective receptors on hepatocytes and transduction of this signal induce changes in APP gene expression that are primarily regulated at a transcriptional level. Under certain conditions post-transcriptional mechanisms, translation, APP modelling and export, may also be involved in this process (Kushner 1993, Pannen & Robotham 1995). Not only the liver is capable of producing APP: many of them are also produced extrahepatically, e.g.  $\alpha_1$ -PI, ceruloplasmin (Cp), complement components, and SAA (Raynes 1994). Glucocorticoids (cortisol) play a major role in modulating the APR. Cortisol enhances the IL-6-mediated APP production. It also reduces the release of pro-inflammatory cytokines, decreases capillary permeability and leucocyte recruitment, stabilizes lysosomal membranes, and suppresses cells of the immune system.

### *Kinetics of plasma APP*

The APP profiles vary among different animal species (Kushner 1982, Hayes 1994) and also within them. The profiles can be affected e.g. by age, sex, pregnancy, and polymorphism (Alsemgeest *et al.* 1993, Hayes 1994). The synthesis, secretion and clearance vary between different APP (Hayes 1994). First APP are produced within a few hours of tissue injury, and their peak values can be reached within one day after the onset of tissue injury (Boosman *et al.* 1989). Constitutively secreted plasma proteins respond with delay followed by sustained responses due to longer half-lives (Hayes 1994). In some inflammatory diseases certain APP may be more actively consumed, thus resulting in relatively low APP levels considering the stage of inflammation (Thompson *et al.* 1992). Not only the concentration, but also the glycosylation stage of plasma proteins change during acute phase (Nagahata *et al.* 1989, Turner 1995). For most APP used in clinical veterinary medicine, kinetics and behaviour in different pathological conditions have yet not been described. Therefore, it is difficult to interpret the significance of a particular level of an induced APP in different animal species (Hayes 1994).

### *Function of plasma APP*

Traditionally, plasma APP have been classified according to their classically known functions and to their structural properties. Recently, knowledge of their function has markedly increased, and several new functions have been discovered for them (Cooper 1990).

### *Pentraxin family*

CRP and serum amyloid-P component (SAP) are members of the highly conserved pentraxin family of plasma proteins with a pentameric character. Pentraxins are able to clear nuclear material released from necrotic tissue; they are also involved in opsonization, activation of classical pathway of complement, chemoattraction, and enhancement of phagocytosis (Cooper 1990, Raynes 1994, Steel & Whitehead 1994, Pannen & Robotham 1995, Tabel 1996). CRP and SAP are major APP in humans, but relatively low responders in cattle (Maudsley *et al.* 1987; Sarikaputi *et al.* 1992).

### *Serum amyloid-A (SAA) family*

Members of the SAA family are small apolipoproteins that associate with a fraction of high-density lipoprotein (HDL) during APR (Cooper 1990, Pannen & Robotham 1995). SAA is considered a major APP in humans and also in cattle (Hayes 1994, Yamamoto *et al.* 1998). It has been speculated that these SAA family proteins are involved in the alteration of cholesterol metabolism under inflammatory conditions (Pannen & Robotham 1995).

### *Metal-binding Proteins*

Metal-binding proteins that increase in plasma concentration during acute phase include e.g. Hp and Cp (Pannen & Robotham 1995). Hp, a moderate APP in humans and a major APP in cattle, is able to bind haemoglobin and thus prevents the loss of iron. This reduction in iron availability might be of importance to resist bacterial infections, as iron is required for the microbial growth. Copper is an integral part of Cp, an APP in humans and in cattle (Hayes 1994). During copper deficiency, Cp concentrations are reduced (Mulhern & Koller 1988). Some members of this group can also function as scavengers of free oxygen radicals. The main function of Hp is considered to be the capability of binding Hb and the transportation of the Hp-Hb complex to the liver. Because of this, during intravascular haemolysis Hp levels typically decrease despite the ongoing APR (Thompson *et al.*, 1992). Hp has also many other properties; it participates in immunological functions, being suggested to have immunosuppressive properties (Murata & Miyamoto 1993). Hp is also involved in erythrocyte aggregation (Weng *et al.* 1997), and in neurological depression (Maes 1993). Not only the concentration of Hp, but its glycosylation type and level have been reported to change during the inflammatory process (Turner 1995).

### *Proteinase inhibitors*

Proteinase inhibitors are able to neutralize lysosomal hydrolases released by phagocyte cells. Several proteins of this group belong to the family of serine protease inhibitors, e.g.  $\alpha_1$ -PI. Alpha<sub>1</sub>-PI rapidly binds to neutrophil elastase, and is therefore considered to be an elastase inhibitor. Alpha<sub>1</sub>-PI is a moderately reacting APP in both humans and cattle

(Conner *et al.* 1986, 1988; Thompson *et al.* 1992). In vasculitis with leucocyte enzyme release the  $\alpha_1$ -PI levels decrease (Thompson *et al.* 1992).

### *Coagulation proteins*

The activation of coagulation cascade in response to tissue injury involves many coagulation proteins. Fb is a protein of this group and its synthesis increases during an APR. It is a coagulation protein serving as a matrix for wound healing (Raynes 1994). Fb is a minor APP in both humans and cattle (Hayes 1994). In the presence of intravascular coagulation, Fb concentrations decrease (Thompson *et al.* 1992).

### *Other Proteins*

The exact physiological function of  $\alpha_1$ -AG is not clear. However, there is some evidence that  $\alpha_1$ -AG might contribute to the net charge on microvessel walls and could decrease albumin leakage from circulation during the acute phase. In cattle,  $\alpha_1$ -AG is known to suppress lymphocyte blastogenesis and thus possess immunosuppressive properties (Motoi *et al.* 1992; Sato *et al.* 1995). Alpha<sub>1</sub>-AG is a moderately reacting APP in cattle (Conner *et al.* 1988).

### *Classification of APP according to their roles in inflammation*

APP can also be classified according to their functions in the inflammatory process. Roles that can be attributed to the majority of the known APP are: mediators (CRP, complement proteins), modulators (complement and clotting pathway inhibitors), inhibitors (protease inhibitors), scavengers (CRP, Hp, SAA), immunomodulators ( $\alpha_1$ -AG), and repairers and resolvers (protease inhibitors,  $\alpha_1$ -AG) (Thompson *et al.*, 1992).

### *Bovine APP*

Despite the uniform nature of the APR, there are numerous differences in the acute phase characteristics between different animal species. The background to this phenomenon is poorly understood. Plasma APP have typically their representatives in different species, but their response patterns can vary greatly. CRP is a good example of this phenomenon: in healthy humans it is practically negligible, but has a high relative increase in bacterial

infections (Steel & Whitehead 1994), whereas in healthy cattle it is present, but does not increase markedly during APR (Kent 1992). In contrast, human Hp is a constitutively secreted plasma protein with only a moderate relative increase during APR, whereas in healthy cattle it is practically negligible, but has a high relative increase during APR.

### *Haptoglobin*

Bovine Hp has two subunits with molecular weights of 20-23 ( $\alpha$ -subunit) and 35-37 kD ( $\beta$ -subunit) (Morimatsu et al. 1992, Yoshino et al. 1992, Godson et al. 1996). In circulation, it is highly polymerized having a molecular weight of approximately 1000-2000 kD (Godson et al. 1996). Bovine Hp exists also as polymers associated with albumin (Eckersall & Conner 1990).

Bovine Hp was first documented by Bremner (1964) who reported that plasma samples from healthy calves contained very little Hp, and that local inflammation induced by injection of turpentine elevated Hp concentrations greatly. Before this, Liang (1957) showed the ability of Hb to bind plasma proteins in cattle. Spooner & Miller (1971) reported that this Hb-reactive protein was detected only in 0.6% of clinically healthy cattle, but in most of the cows with diagnosed bacterial diseases. Blackshaw (1979), Makimura & Suzuki (1982), Conner et al. (1986), Eckersall & Conner (1988), Conner et al. (1989) and Skinner et al. (1991) reported Hp to be a useful marker for detecting bovine bacterial infections. Alsemgeest et al. (1994) found a significant difference in Hp levels ( $P < 0.001$ ) between healthy animals and animals with inflammatory diseases similar to this study; Hp was increased particularly in chronically diseased animals. Godson et al., (1996) and Young et al. (1996) found Hp to be a valuable diagnostic aid in bovine respiratory disease (BRD), and Wittum et al. (1996) suggested Hp to indicate response of BRD to antimicrobial therapy. Hp has also been reported to increase at the viraemic stage of foot-and-mouth disease (Höfner et al. 1994). It has also been found in serum of cows at parturition (Uchida et al. 1993), in cows with fatty liver (Nakagawa et al. 1997) and in plasma of bull calves after surgical castration (Fisher et al. 1997). We previously reported bovine Hp to be a major bovine APP having a high relative increase during APR in dairy cows with experimentally induced *E. coli* mastitis (Salonen et al. 1996). Bovine Hp

response has also been studied in vitro: calf liver parenchymal cells have been reported to release Hp after treatment with dexamethasone (Higuchi et al. 1994), and primary bovine hepatocytes secrete Hp after stimulation by pro-inflammatory cytokines IL-6 and TNF- $\alpha$  (Alsemgeest et al. 1996). According to Richter (1974), plasma Hp concentration is not significantly affected by gender, pregnancy, lactation status, or age of an animal.

Bovine serum Hp has been traditionally analyzed indirectly by measurement of Hb bound to Hp (Makimura & Suzuki 1982, Salonen et al. 1996). Morimatsu et al. (1992) introduced a single radial immunodiffusion assay for bovine serum Hp. Recently, monoclonal antibodies against bovine Hp have been characterized and used for analysing bovine serum Hp by several immunotechniques (Sheffield et al., 1994; McNair et al., 1995; Young et al., 1995; Saini et al., 1998).

#### *Serum amyloid-A*

Yamamoto *et al.* (1998) purified a bovine 14 kD SAA which was associated with a fraction of HDL. This 14 kD SAA increased in serum of calves experimentally infected with *Pasteurella haemolytica*, whereas two low molecular mass proteins immunologically related to the 14 kD protein were conversely decreased. According to Alsemgeest *et al.* (1995), SAA consists of multiple isoforms which occur in different plasma concentration ratios during different bovine diseases. In cattle, SAA is a major APP with high sensitivity to inflammatory challenge (Gruys *et al.* 1993, 1994). SAA has a low constitutive level in plasma, and the relative increase during an APR can be over 10-fold (Hayes 1994). In cattle, SAA has been reported to increase in various inflammatory conditions: in calves after intra-tracheal inoculation with *P. haemolytica* (Horadagoda *et al.* 1993, 1994), after physical stress in calves and after calving in cows (Alsemgeest *et al.* 1993, 1995b), after surgery (Alsemgeest *et al.* 1992), as well as after endotoxin administration (Boosman *et al.* 1989, Alsemgeest *et al.* 1992, Werling *et al.* 1996). Bovine SAA increases also during the peripartum period in maternal serum (Alsemgeest et al., 1993). In a study with clinically diseased cattle, SAA concentration was increased in acutely, subacutely and chronically diseased animals (Alsemgeest *et al.* 1994). Bovine SAA can be analyzed immunologically,

and enzyme-linked immunosorbent assays (ELISA) for the determination of bovine SAA have been developed (Boosman *et al.* 1989; Horadagoda *et al.* 1993).

### *Fibrinogen*

Bovine Fb is a constitutive plasma protein with a moderate increase during an APR (Hayes 1994). It increases in various inflammatory conditions of cattle, such as peritonitis, endocarditis, pericarditis, pneumonia, and nephritis (McSherry *et al.*, 1970; Sutton & Hobman, 1975). In calves, *E. coli* challenge and turpentine injection trigger a Fb response (Deldar *et al.*, 1984; Conner *et al.*, 1988). However, plasma Fb concentration can also remain unchanged or decrease during acute inflammatory conditions of cattle. This may reflect consumption of the protein at the inflamed area which transiently can exceed the production (Welles *et al.* 1993). Therefore a low plasma Fb value in a diseased animal can be a poor prognostic sign (McSherry *et al.* 1970). According to Holst & Svensson (1994), an experimental oral infection of calves with *Eimeria alabamensis* does not alter plasma fibrinogen concentration. Copper deficiency has been reported to alter the Fb response of beef heifers to bovine herpesvirus-1 (Arthington *et al.* 1996).

### *Acid soluble glycoproteins*

Alpha<sub>1</sub>-acid glycoprotein (formerly known as seromuroid) is a constitutive plasma protein which has a moderate and relatively slow response after a tissue injury in cattle (Conner *et al.* 1988, 1989, Motoi *et al.* 1992). Its basic function is not clear, although it is known to possess immunoregulatory properties, e.g. suppression of lymphocyte blastogenesis (Motoi *et al.* 1992, Sato *et al.* 1995). Alpha<sub>1</sub>-AG is known to increase in several diseases of cattle: traumatic pericarditis, arthritis, mastitis, pneumonia (Tamura *et al.* 1989), and after subcutaneous inoculation of *P. haemolytica* in calves (Walker *et al.* 1994). In adult cattle with hepatic abscesses or enzootic bovine leucosis (EBL),  $\alpha_1$ -AG activity was particularly intense in hepatocytes adjacent to abscesses or EBL-induced tumors (Itoh *et al.* 1997). Bovine  $\alpha_1$ -AG has traditionally been analyzed using an acid precipitation technique (Conner *et al.* 1988). Immunotechniques for the quantitative analysis of bovine  $\alpha_1$ -AG include single radial immunodiffusion method (Tamura *et al.* 1989), and nephelometric and turbidometric immunoassay methods (Komine *et al.*, 1994). A well-known property of  $\alpha_1$ -

AG is its ability to bind cationic drugs (Kremer et al. 1988, Son et al. 1996). This may be of clinical pharmacological importance e.g. in neonatal calves, because serum  $\alpha_1$ -AG concentration peaks in them (Itoh et al. 1993).

As many of the APP are glycoproteins, estimation of serum glycoprotein level can provide information about the APR status. A method for a rapid analysis of serum acid soluble glycoproteins (ASG) has been described (Nakajima et al. 1982). The components of bovine ASG have not been exclusively identified, but  $\alpha_1$ -PI and  $\alpha_1$ -AG are presumably involved. Using this method, Nagahata et al. (1989) found elevated ASG concentrations in enzootic bovine leucosis, in cows with acute clinical mastitis, and in cattle after surgery.

#### *Alpha<sub>1</sub>-proteinase inhibitor*

Alpha<sub>1</sub>-PI (also known as  $\alpha_1$ -antitrypsin,  $\alpha_1$ -AT) is a serine protease inhibitor with a moderate relative increase during APR in cattle. It is mainly produced in the liver with minor expression in tissue macrophages (Roberts *et al.* 1995). Alpha<sub>1</sub>-PI plays a major role in protecting the host from the activities of neutrophil elastase (Roberts *et al.* 1995). During mastitis,  $\alpha_1$ -PI is actively transported to the mammary gland, and is well-described as an indicator of bovine mastitis (Honkanen-Buzalski *et al.* 1981, Honkanen-Buzalski & Sandholm 1981, Conner *et al.* 1986). Alpha<sub>1</sub>-PI has also been reported to increase after turpentine injection of calves (Conner *et al.* 1988), after experimental infection of calves with *P. haemolytica* and *Ostertagia ostertagi*, and in calves after intravenous administration of endotoxin (Conner *et al.* 1989). Several methods have been developed for the qualitative and quantitative analysis of  $\alpha_1$ -PI, including a functional analysis of trypsin-inhibitory capacity (TIC) of  $\alpha_1$ -PI (Sandholm *et al.* 1984). It must be noted that the functional and immunological analyses can provide different results, because the plasma concentration of functionally active and total  $\alpha_1$ -PI is not necessarily the same.

#### *Other bovine APP*

Several other plasma proteins also respond during APR in cattle. Of these, Cp, has been shown to increase in mastitis (Conner *et al.* 1986), after experimental infection with *Salmonella dublin* (Piercy 1979), after turpentine infection of calves (Conner *et al.* 1988),



and after intranasal inoculation of beef heifers with bovine herpesvirus-1 (Arthington *et al.*, 1996). Alpha<sub>2</sub>-macroglobulin, a proteinase inhibitor, has been reported to increase in plasma following infection with *P. haemolytica* (Conner *et al.* 1989, Cheryk *et al.* 1998), and in mastitic milk (Rantamäki & Müller 1992). Bovine lipopolysaccharide binding protein and its increase after intratracheal inoculation of calves with *P. haemolytica* type A has been characterized in cattle (Horadagoda *et al.* 1995, Bochsler *et al.* 1996). CRP and SAP, known as APP in humans, have also been isolated and characterized from bovine serum (Maudsley *et al.* 1987, Morimatsu *et al.* 1989), but do not respond markedly during APR (Maudsley *et al.* 1987, Akiyama *et al.* 1992). Lactating cows have been reported to have higher serum CRP and SAP levels than non-lactating ones (Morimatsu *et al.* 1991). Unlike in serum, CRP concentration increases in bovine milk during mastitis, and has been introduced as an inflammatory marker for controlling udder health (Schrödl *et al.* 1995).

*Table 3. Illustration of bovine plasma APP according to their responsivity during APR.*

Major APP (10-100 fold increase)	Haptoglobin (Hp) Serum amyloid-A (SAA)
Moderate APP (2-10 fold increase)	$\alpha_1$ -acid glycoprotein ( $\alpha_1$ -AG) $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ -PI)
Minor APP (1-5 fold increase)	Fibrinogen Ceruloplasmin $\alpha_2$ -macroglobulin ( $\alpha_2$ -M) Complement component 3 (C3) Bovine lipopolysaccharide binding protein (bLBP)

## **Diagnostics of bovine inflammatory diseases**

### *Present situation*

There is only a limited number of haematological tests for the practicing veterinarian to diagnose bovine inflammatory diseases. In the field, the diagnostics are based on the case history and physical examination of the animal.

### *White blood cell count*

White blood cell (WBC) count is a fundamental tool when diagnosing inflammatory/infectious diseases in most animal species. It is not a very accurate method

for that purpose in ruminants because the total WBC count rarely increases very strongly (Kidd 1991, Cole *et al.* 1997). Instead, the differential WBC counts are considered more informative in cattle. Cows respond to acute inflammation with initial leucopenia and a degenerative left shift. The leucopenia can be explained by stress-induced loss of lymphocytes, which are the most numerous subspecies of leucocytes in the cows, by the migration of neutrophils to the inflamed area, and by the only marginal release of mature neutrophils from the bone marrow (Kidd 1991, Jain 1993, Cole *et al.* 1997). Conventional haematology has been used e.g. to follow-up of bovine patients after surgery (Poulsen 1974, Hjortkjær & Svendsen 1979).

#### *Erythrocyte sedimentation rate*

Erythrocyte sedimentation rate (ESR) is an old measure of inflammatory conditions in humans showing prominent rouleaux formation and rapid sedimentation. Bovine erythrocytes act differently and do not form rouleaux or sediment markedly in healthy or diseased animals; therefore they lack diagnostic value (Jain 1993).

#### *Platelet count*

Thrombocytopenia is a well-recognised finding during the acute phase of bacterial infections in the cow (Deldar *et al.* 1984, Welles *et al.* 1993, Cheryk *et al.* 1998). The diagnostic significance of thrombocytosis has not been widely verified. According to Hawkey *et al.* (1990), platelet count could be of more use than WBC for identifying and following the course of bacterial infections in bovine species in which the WBC response to infection is minimal: an increased platelet count would indicate the persistence of a bacterial infection. This postulate has not been supported by the results from further clinical studies.

#### *Gammaglobulin concentration*

Increase of blood gammaglobulin levels is a typical phenomenon in chronic infections (Jain 1993). Sandholm (19974) developed a semiquantitative glutaraldehyde test for simultaneously increased gammaglobulin and Fb concentration. The test is used in differential diagnostics of purulent infectious diseases in cattle. The advantage of this test is

that it uses whole blood which makes it practical for field conditions as a cow-side test (Sandholm 1974b).

#### *APP in bovine clinical diagnostics*

A plasma protein having a low basal level, a rapid response, a high relative increase and a rapid clearance, is most suitable for diagnostic use. Concentrations of an optimal APP also correlate well with the degree of tissue damage and with the recovery after treatment (Kent, 1992). In bovine medicine, there are several areas where APP could be implicated.

#### *APP as a clinical tool*

##### *Clinical diagnostics*

The most obvious indication for use of APP is clinical diagnostics. First, APP could serve as indicators for subclinical diseases with individual animals or within herds. In clinical cases, they could provide additional information and thus improve diagnostics and help setting prognosis. Scott *et al.* (1992) reported serum Hp to have prognostic value in ovine dystocia cases, where serum Hp concentration of above 1.0 g/l indicated a reduced survival rate. Eckersall *et al.* (1988) found Hp a useful prognostic indicator in cattle: in diseased animals with Hp values between 0.1 and 1.0 g/l of Hb-binding capacity (HbBC) the prognosis was guarded, and when the Hp values were >1.0 g/l HbBC the prognosis was poor. APP may also serve in the follow-up of medical treatment, where sequential APP determinations would provide accurate information of the course of the disease. The concentration of plasma Fb has been monitored during the follow-up of equine patients after surgery (Allen & Kold 1988). As dairy units become larger, less time is spent with clinical examination of an individual animal. For this reason, additional information provided by diagnostic tests, like APP determinations, can play a role in decision making with individual animals.

#### *APP in the meat industry*

One potential indication for the use of APP is to improve the quality of the meat inspection process. According to Saini & Webert (1991), incorporation of APP tests to *ante mortem* and *post mortem* inspection process would yield valuable information. It would allow

screening of all animals to identify those with disease activity, confirm presence of disease in suspect animals at *ante mortem* inspection, and confirm the presence of a systemic illness at *post mortem* inspection. For these purposes, the non-specific nature of the APP is a major advantage (Saini & Webert 1991; Burger *et al.*, 1992; Eckersall *et al.*, 1992; Eurell *et al.*, 1992; Hall *et al.*, 1992; Visser *et al.*, 1992). Public health is another concern affecting the introduction of APP tests to the meat industry. Control of microbes that are able to create food-borne epidemics, like *Salmonella*, *Listeria*, *E. coli*, *Toxoplasma*, and *Campylobacter* are of specific interest. Furthermore, traditional meat inspection methods are not effective in detecting some other diseases, like tuberculosis or cysticercosis. On-line APP tests would improve the sensitivity of traditional meat inspection protocols and prevent the contamination of meat processing plants (Saini *et al.* 1998).

#### *APP in medical science*

APP could also serve as inflammatory markers in medical science. APP could provide accurate information about the health status of experimental animals, and about the pathophysiological and pathogenetical events during an experimental study with animals or their tissues. Pharmacological studies would also benefit APP determinations: they could be used for example when response to vaccines is studied (Stokka *et al.* 1994).

#### *Animal welfare*

APP have potential as indicators of stress (Alsemgeest *et al.* 1992). From this point of view, they could serve as objective and quantitative indicators of animal welfare in livestock production and in medical science using experimental animals.

#### *APP as markers for disease resistance*

As mentioned earlier, APP play a significant role in resisting infectious diseases by several mechanisms.

In certain infectious diseases, APP levels have been found to differ between resistant and sensitive animals. For example, mice resistant to African trypanosomiasis possess much higher Hp response than the sensitive ones (Shapiro & Black 1992). APP have thus been proposed to serve as non-specific resistance markers for infectious diseases (Table 4.).

*Table 4. Possible indications for the use of acute phase proteins in veterinary medicine.*

Clinical diagnostics	Detection of subclinical infections Assessment of the severity of disease Differential diagnostics of bacterial and viral diseases
Prognostic marker for treatment response and follow-up of treatment	Anti-inflammatory treatment Antimicrobial treatment Antiparasitic treatment Surgical treatment
Quality control in meat industry	Detection of latent and subclinical infections Quantitative marker for the disease severity
Medical science	Pathogenetical study Pathophysiological study Pharmacological study
Animal welfare	Stress indicator
Non-specific marker for disease resistance	Malaria Babesiosis Trypanosomiasis Streptococcal infection

The characteristics of bovine APP are still poorly understood. The most important questions to be answered concern the functions of individual proteins, their responsiveness and kinetics in various inflammatory conditions, the methodological questions considering their analysis from biological samples, as well as their diagnostic and prognostic value in veterinary medicine.

In veterinary, as well as in human medicine, it is essential to understand the pathophysiology of the inflammatory response of the host. This is especially the case in infectious diseases where it is usual that the signs of inflammation are considered as the signs of infection, which is not necessarily true. For this reason, understanding the APR is important in setting diagnosis and making the decision on whether to start e.g. antimicrobial treatment. A more accurate diagnosis and distinguishing between viral and bacterial diseases would also decrease unnecessary use of antimicrobials.

It has also been stated that antimicrobials are more effective during the acute phase of an infection than in its subclinical phase. An obvious reason for this is that APR participates in bacterial killing and thus improves the effect of antimicrobial treatment. This “synergism”

is still poorly understood and should be exploited more in both human and veterinary medicine.

## **AIMS OF THE STUDY**

Although a number of APP have already been characterized in the cow, little is known about their role in different clinical conditions. The aims of the present study were to:

Study the characteristics of bovine APR in various clinical diseases of dairy cattle.

Study the diagnostic capacity of bovine APP as disease markers in various clinical diseases of dairy cattle and compare it with the other diagnostic markers used in bovine medicine.

Study the prognostic value of bovine APP for predicting the course of disease, survival of the animal, productive capacity, fertility, and/or expected meat inspection result in various clinical diseases of dairy cattle.

## **MATERIALS**

### **Animals**

#### *Heifers with experimentally induced aerobic-anaerobic mastitis (I)*

Ten pregnant heifers expected to calve in one to two months were used. Both hind quarters of each heifer were experimentally challenged with a combination of *Actinomyces pyogenes*, *Fusobacterium necrophorum* and *Peptostreptococcus indolicus*. The induction of mastitis and follow-up of the experimental animals was carried out as described by Hirvonen *et al.* (1994). Development of mastitis was monitored by assessment of systemic and local clinical signs, by bacteriological examination of udder secretion samples, and by blood leucocyte count and a panel of serum APP. The outcome of the experimental mastitis was assessed after calving. The experiment was approved by the Animal Experimentation Committee of the College of Veterinary Medicine, Helsinki, Finland.

#### *Dairy cows with experimentally induced E. coli mastitis (IV)*

Six clinically healthy, early lactating cows (median lactation day 29) were used in the study. The cows produced low somatic cell count (SCC) milk of <100,000 cells/ml with a mean of 17 l of milk/d (range 12-28 l). The cows were challenged with 1500 CFU of *E. coli* FT238 strain into a single udder quarter. The strain had been isolated from a cow suffering from clinical mastitis, and was nonhemolytic, intermediately serum resistant, and in vitro sensitive to enrofloxacin (MIC < 0.25 mg/ml). The cows were first challenged in one udder quarter, and 3 weeks later in the contralateral quarter. Systemic and local clinical signs were recorded throughout the experiment. Milk samples were collected for bacteriological analysis and for analysis of inflammatory indicators of milk. Serum samples were obtained from the jugular vein before bacterial challenge and sequentially thereafter. Cows were randomly allocated into two treatment regimes. At the initial challenge, all 6 cows received a single systemic flunixin meglumine treatment. Three of the animals also received systemic enrofloxacin for 3 days. Treatment began 12 hours after the bacterial challenge. At the 2<sup>nd</sup> challenge 3 weeks later, treatments were changed vice versa. The experiment was approved by the Animal Experimentation Committee of the College of Veterinary Medicine, Helsinki, Finland.



*Dairy cows with acute postpartum metritis (V)*

A total of 29 Holstein-Friesian cows from one Hungarian large scale dairy farm were studied. Of these, 19 cows suffered from acute metritis, and 10 cows served as controls. During a postpartum period of 50 days, the cows were examined clinically and by vaginoscopy, rectal palpation, and uterine bacteriology. The recorded clinical signs included appetite, rectal temperature, and appearance of vaginal discharge. Signs of estrus were observed twice daily, and the animals were vaginoscoped for detection of the cervical discharge. Rectal palpation was used to evaluate the status of uterus. Uterine bacteriology was examined from uterine swab samples with aerobic and anaerobic culturing. Concomitant diseases, including metabolic disorders and clinical mastitis, were also recorded. Blood samples were collected from the coccygeal vein every second day for analysis of plasma progesterone and APP concentrations. As part of a larger experiment, the 19 cows with acute metritis received intrauterine antibiotic treatment at 4-11 days after calving. Three animals were further treated with systemic administration of antibiotics.

*Dairy cows with surgically treated abdominal disorders (II)*

Ninety seven cows suffering from abdominal disorders were submitted for surgery to the Large Animal Clinic of the Faculty of Veterinary Medicine of University of Helsinki during 1993. Eleven cows were suffering from traumatic reticuloperitonitis, 67 from abomasal displacement or volvulus, 10 from other gastrointestinal disorders, and nine cows from dystocia. The cows were operated on either during the day of arrival or on the following day; all caesarean sections were performed on the arrival day. Blood samples for the haematological analyses were taken from the jugular vein immediately after admission to the clinic and at 1-2 day intervals thereafter. Additional plasma samples for APP analysis were collected at admission and 1-2 times after surgery. After surgery, all cows were treated systemically with penicillin G for 3-5 days with supportive treatment if needed. The cows were usually hospitalized for 8 days and then returned to their farms. The cows that developed complications were hospitalized for up to 2 weeks for further treatment.

*Emergency slaughtered dairy cows (III)*

Eighty emergency slaughtered Ayshire or Friesian dairy cows were included in the study. Thirty percent of the cows had calved within one week and 60% within two months before culling. Blood samples for serum protein and enzyme analyses were obtained from the jugular vein at exsanguination of each cow. The meat inspection data for each animal were collected. They consisted of case history and pathological diagnosis based on the meat inspection results, including the amounts of acceptable and condemned meat. The weights of acceptable and condemned meat were measured under control of the veterinarian responsible for the meat inspection.

## **METHODS**

### **Collection of blood samples**

Samples for haematological analyses were collected in EDTA-containing plastic tubes. Other plasma samples were collected from the jugular or coccygeal vein in plain tubes with 0.1 mol/l sodium citrate (9 ml of blood and 1 ml sodium citrate). Plasma from citrated tubes was separated by centrifugation at 1000 g for 15 minutes and stored at -70°C until analyzed. Serum samples were collected in plain glass tubes and further treated as mentioned above.

### **Haematology (I, II)**

The haematological analyses were carried out with a Coulter Counter (M530, Coulter Electronics Ltd., Luton, UK) particle counter. Hb concentration, red blood cell count, and total leucocyte count were recorded. RBC and mean corpuscular volume (MCV) were used to calculate the PCV, which is often referred to as the "haematocrit". Differential leucocyte counts were achieved by staining of blood smears with May-Grünwald-Giemsa and by sequential counting 200 cells under a microscope using 50-fold magnification.

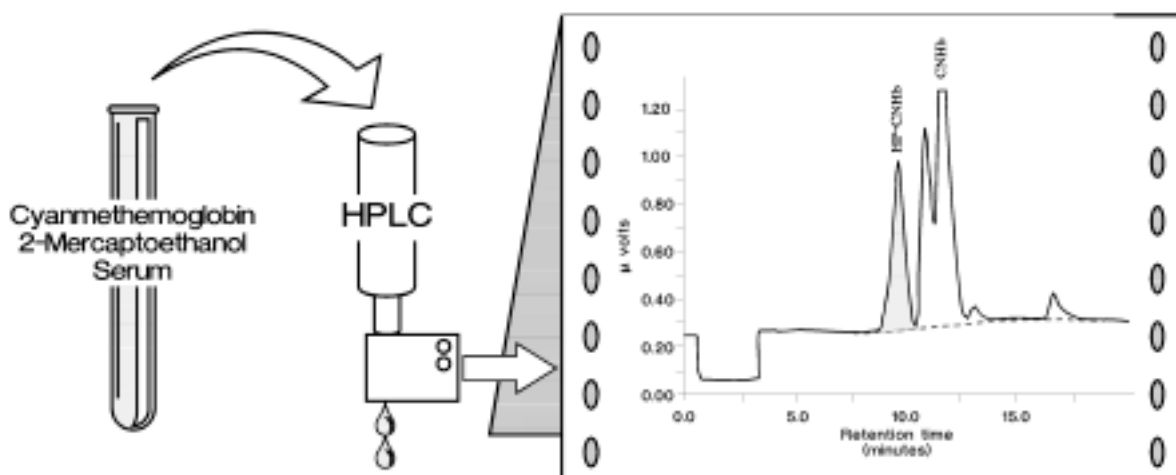
### **Analysis of APP**

#### *Plasma and serum Hp analysis*

#### *High performance liquid chromatography method (III and IV)*

Serum Hp was analyzed using a High Performance Liquid Chromatography Method (HPLC) developed at our laboratory for this purpose (Salonen *et al.* 1996). The method is based on binding of depolymerized bovine Hp on excess bovine cyanmethemoglobin (HbCN) and separating the Hp-cyanmethaemoglobin (Hp-HbCN) complex and free HbCN by gelfiltration on HPLC. Stock HbCN solution was prepared from washed bovine red blood cells. Ten ml of citrated blood was centrifuged and the packed cells washed three times with 0.9% saline. One ml of packed cells was hemolyzed with 9 ml of distilled water. The lysate was diluted with Drabkin's solution to give an Hb concentration of 0.6 g/l, as measured by spectrophotometry at 540 nm. To register a distinct Hp-HbCN peak during HPLC, Hp was depolymerized by pretreating the serum samples with 0.1M 2-

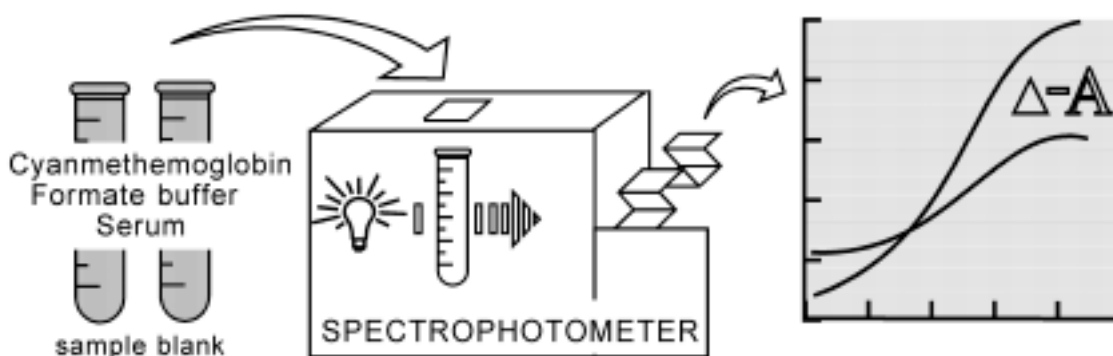
mercaptoethanol diluted in saline. One part of the serum sample was mixed with one part of 2-mercaptoethanol and two parts of HbCN. Ten  $\mu$ l of the mixture was injected into TSK PWXL guard column (6mm x 40mm) and TSK G5000 gel filtration column (5 mm x 300 mm) (TSK, Tosoh Co, Japan) equilibrated with 50 mM phosphate buffer in pH 6.5 (flow rate 1.0 ml/min). A Waters HPLC-system with type 600E system controller with pump, type 994 diode array detection, WISP 700 autosampler and Baseline 810 analyzing software were used (Waters Associates Inc.). The Hp-HbCN complex was eluted before serum protein and the free HbCN and was scanned. The absorption of the complex peaked at 398 nm, and this wavelength was used in subsequent assays. The HPLC method was standardized with a known amount of human haptoglobin (S-Haptog, Impro-Vakio, Bioclin, Helsinki) assuming a direct relationship between human and bovine serum Hp concentrations (g/l). The detection limit of the HPLC assay was < 0.005 g/l. Samples containing > 1.0 g/l of Hp were diluted to a concentration falling within the range of the standard curve. The sensitivity of the HPLC method to haemolysis was tested by adding free Hb to the serum samples. Haemolysis was found not to interfere with the method. The intra-assay coefficient of variation was less than 2%.



*Figure 1. The principle of the HPLC method for the analysis of Hp from bovine serum samples. The method is based on binding of depolymerized bovine Hp on excess bovine HbCN and separating the Hp-HbCN complex and free HbCN by gel filtration on HPLC. The complex is detected photometrically at the absorption peak of 398 nm.*

### *Photometric method (I, II, V)*

Plasma Hp was analyzed photometrically as described by Elson (19974) with slight modifications. The analysis is based on the observation that Hb, when bound to Hp, is protected from denaturation in acidic medium (formate buffer, pH 3.7) (Tarukoski 1966). The absorption of the acidified Hp-HbCN complex was measured at 405 nm (Harvey 1976) and 380 nm; the latter is the isospeptic point of the absorption curves of the sample and blank. A Hitachi Model U 2000 UV/VIS Spectrophotometer (Hitachi Ltd.) was used. The Hb-binding capacity (HbBC, unit g/l) of plasma Hp was calculated using the differential extinction coefficient of the complex and free HbCN. The intra-assay coefficient of variation was 10%. The sensitivity of the photometric method to haemolysis was tested similarly as with the HPLC assay. Haemolyzed samples interfered with the method, and were excluded from the analyses.



*Figure 2. The principle of the photometric method for the analysis of plasma Hp. Hp-HbCN complex protects HbCN from denaturation in acidic medium. The complex is detected photometrically at 405 and 380 nm; the latter is the isospeptic point of the absorption curves of the sample and blank.*

### *Plasma Fb Analysis*

#### *Photometric Method (I)*

The method was based on the photometric determination of the thrombin induced rate of plasma clotting. An excess of thrombin was added to diluted plasma samples. The increase in turbidity showed a linear relationship with plasma Fb contents. Bovine thrombin (Topostasine<sup>R</sup>, Hoffmann-La Roche) was dissolved in barbital buffer (0.02 M, pH 8.6) resulting in a thrombin solution with 30 N.I.H. units/ml. The plasma samples were diluted 1:8 with the

barbital buffer. An equal volume of diluted samples and the thrombin solution were mixed at +37°C. The increase in turbidity was measured between 2 and 60 seconds at 340 nm. A kinetic programme was used to interpolate the Fb levels (g/l) from the standard curve. Fb standard was prepared from plasma of known Fb level, as described by Clauss (19957).

#### *Heat Precipitation Method (II)*

Fb was determined semiquantitatively using the routine heat precipitation technique (56°C) after Millar (Millar 1971). First, blood is collected in EDTA tubes and whole blood is drawn into a microhematocrit tube (diameter 1.5-1.6 mm, length 75 mm, volume 75 (l) and sealed at one end. The tube is spun for 5 minutes in a microhaematocrit centrifuge. Fb is then precipitated by placing the tube in water-bath at 56°C for 3 minutes. The precipitated Fb is packed on top of the buffy coat by further centrifugation for 3 minutes. The length of the column of the packed precipitate is measured in relation to that of the length of the total plasma column. Reading of the column is facilitated by using a microscope with an ocular micrometer. The plasma concentration of Fb is calculated by multiplying the above mentioned relation by 100; this gives Fb concentration as g/l.

#### *SAA analysis (IV)*

Serum SAA concentration was measured by radial immunodiffusion using antiserum to human amyloid-A as described earlier (Maury & Teppo 1984). Purified human amyloid-A protein was used as a standard. The detection limit of the assay is 5 mg/l for human SAA.

#### *Plasma ASG analysis (I)*

Serum ASG concentration was analyzed photometrically as described by Nagahata *et al.* (1989) with slight modifications. The serum samples were mixed with 0.6 M perchloric acid and centrifuged at 1600 g for 20 minutes. An aliquot of supernatant containing ASG was stained with Coomassie brilliant blue G-250. The serum ASG concentrations were determined spectrophotometrically at 590 nm using bovine serum albumin as a standard protein. The ASG results were expressed as g/l.

### *Plasma and serum $\alpha_1$ -AG analysis (III, V)*

Serum  $\alpha_1$ -AG (g/l) was analyzed using a commercial radial immunodiffusion kit (Saikin Kagaku Institute Co., Ltd., Sendai, Japan). Purified bovine  $\alpha_1$ -AG ( at vials of 250 mg/l and 1000 mg/l) was used as standard protein; the values were expressed as mg/l.

### *Alpha<sub>1</sub>-PI analysis (I)*

Serum  $\alpha_1$ - PI capacity was measured photometrically according to the method of Fritz *et al.* (19974). An excess of trypsin was mixed with the serum sample, resulting in the formation of stable inactive complexes due to trypsin-inhibitors. The remaining trypsin-excess was then measured using a synthetic substrate, *N*-benzoylarginine-*p*-nitroanilide (BAPNA) (Merck). Hydrolysis of the substrate is followed directly by the increase in absorption at 405 nm. Serum samples were pretreated with PEG-6000 to remove the disturbing factors ( $\alpha_2$ -macroglobulin-bound trypsin) having proteinase activity against BAPNA (Sandholm *et al.*, 1984). Because there were considerable variations between the pre-challenge  $\alpha_1$ -PI capacity levels of individual heifers, the results were expressed as percentage changes relative to the day 0  $\alpha_1$ -PI capacity value of the same heifer to make the alterations following bacterial inoculation more readily demonstrated.

### **Other serum analyses**

#### *Plasma and serum protein analysis (II, III)*

Plasma and serum total protein concentrations (TP) were analyzed by the biuret method (Weichselbaum 1946) using a commercial kit (Boehringer Mannheim GmbH, Mannheim, Germany) and albumin concentrations with the immediate bromcresol green reaction (Dumas *et al.* 1971, Gentry & Lumsden 1978) (Albumine-Kit, BioMérieux SA, Lyon, France). An automatic analyser (KONE Specific, Kone Instruments Corp., Espoo, Finland) was used for the determination. Plasma globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration. Serum gammaglobulin concentration was determined by agarose gel electrophoresis (Serum protein electrophoresis, Paragon<sup>R</sup> electrophoresis system, Beckman Instruments Inc., Fullerton, California, USA). Plasma protein:Fb ratio was used in distinguishing hyperfibrinoemia caused by disease from that associated with dehydration. The ratio is

obtained by subtracting Fb from the total plasma protein concentration and then dividing the remainder by the concentration of Fb.

#### *Serum NO<sub>2</sub>/NO<sub>3</sub> analysis (IV)*

Serum NO<sub>2</sub>/NO<sub>3</sub> concentration was analyzed essentially as described by Verdon *et al.* (1995). The method is based on photometric detection of the NO<sub>2</sub>-sulfanilamide complex after addition of  $\alpha_1$ -naftyl-1-diethylenamide at absorbance wavelength of 450 nm, commonly known as Griess reaction. NO<sub>3</sub> was first converted enzymatically to NO<sub>2</sub> by nitrate reductase, and the bulk NO<sub>2</sub> was analyzed as mentioned above. Before analysis, serum samples were first deproteinized by ultrafiltration with regenerated cellulose membrane filters (Ultrafree<sup>R</sup>-MC 10000 NMWL Filter Unit, Millipore).

#### *Serum urea and creatinine analysis (IV)*

Serum urea concentration was determined by an enzymatic, kinetic method (Gutmann & Bergmeyer 1974) using glutamate dehydrogenase as indicator enzyme. Serum creatinine concentration was determined by alkaline picrate without deproteinization (Fabiny & Ertigshausen, 1971). A selective chemistry analyzer (KONE Specific, Kone Instruments Corp.) was used for both determinations.

#### *Serum ASAT and CK activity analysis (III)*

Serum aspartate aminotransferase and creatine kinase activities were determined according to recommendations of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1994, 1979) using commercial kits (Kone Instruments Corp.).

### **Bacteriology**

#### *Heifers with experimentally induced aerobic-anaerobic mastitis (I)*

Development of mastitis was monitored by bacteriological examination of udder secretion samples. The bacteriological samples were taken first at 32 hours after bacterial challenge and serially thereafter. The samples were cultured aerobically and anaerobically as described by Hirvonen *et al.* (1994).



#### *Dairy cows with surgically treated abdominal disorders (II)*

Secondary mastitis was detected in 20% of the surgically treated cows. Isolated bacteria were identified according to standard procedures (Griffin *et al.* 1987).

#### *Dairy cows with experimentally induced E. coli mastitis (IV)*

Development of mastitis was monitored by collecting milk samples from the challenged quarters prior to challenge, and serially from 12 hours after bacterial challenge. Milk bacterial counts were determined by plate count method.

#### *Dairy cows with acute postpartum metritis (V)*

In the study of acute postpartum metritis, uterine swab samples from all cows were collected at the beginning of the examination period, and serially thereafter. Uterine bacteriology was examined from uterine swab samples with aerobic and anaerobic culturing as described by Dohmen *et al.* (1995).

#### *Other milk analyses (I, V)*

The daily milk yield was recorded for the cows with experimentally induced *E. coli* mastitis. In heifers with experimental anaerobic mastitis, the outcome was assessed by measuring daily milk yield from each quarter twice at one week intervals after calving. In cows with experimental *E. coli* mastitis, indirect indicators for mastitis, milk somatic cell counts (SCC) and N-acetyl- $\beta$ -D-glucosaminidase (NAGase) activity (Kitchen 1981), were also measured. Milk SCC was measured with a Fossomatic instrument, and NAGase activity with a commercial milk NAGase test kit (Applied Diagnostics Corp., Helsinki).

### **Statistics**

The following statistical methods were used:

Chi-square test (V)

Descriptive statistics (all articles)

Pearson's correlation analysis (III)

Receiver operating characteristics (ROC) analysis (III)

Repeated measures analyses of variance (IV)

Spearman's rank correlation analysis (III)

Student's *t*-test (all articles)

The computer software Excel 5 (Microsoft Co., Redmont, WA, USA), Nonparametric Receiver Operating Characteristics Analysis, Version 2.5 (Vida S., Montreal General Hospital, Quebec, Canada), Prism 2.0 (Graphpad Software Inc., San Diego, CA, USA), SAS (SAS Institute Inc., Cary, NC, USA), and Statgraphics 2.6 (Manugistics Inc., Rockville, MD, USA) were used.

## RESULTS

### Dairy Heifers with experimentally induced aerobic-anaerobic mastitis (I)

#### Clinical signs

The experimental infection with *Actinomyces pyogenes*, *Fusobacterium necrophorum* and *Peptostreptococcus indolicus* produced a moderate to severe clinical mastitis in all ten heifers. The heifers fell into two categories according to their clinical condition. Four heifers became only temporarily affected while the other six heifers developed a chronic purulent mastitis. Body temperature of the four recovered heifers returned to normal within 24 hours after bacterial inoculation, while in the six non-recovered heifers it remained high for two or three days (Figure 3a). The six non-recovered heifers also showed prolonged local clinical signs, the inoculated quarters being almost blind after calving.

#### APR

Plasma Hp levels started to increase within the first day after the bacterial challenge, and the maximum values were reached in two or three days. All heifers responded to the challenge with increased Hp. The Hp response of the six non-recovered heifers was, according to the maximum Hp values, four times higher than that of the other four heifers; the difference was statistically significant between 2 and 5 days after bacterial challenge ( $P < 0.01-0.001$ ). The Hp levels of the six heifers also remained elevated for even two weeks after inoculation, while the four recovered heifers returned to normal in five days after the bacterial challenge (Figure 3b).

Plasma Fb concentration started to rise within the first two days after the bacterial challenge, and the maximum values were reached in two to five days. All heifers responded to the bacterial challenge by increased Fb, the average increase being approximately 2-fold. The acid soluble glycoprotein (ASG) levels started rising within two days after the bacterial challenge. The increase was slow, and the maximum values were reached in three to five days. The ASG response of the six non-recovered heifers was approximately 2-fold, while the recovered four heifers showed only a very mild response; the difference was statistically significant between 3 and 7 days after bacterial challenge. ASG values of the

six heifers remained high for up to 2 weeks after inoculation (Figure 3d). Serum  $\alpha_1$ -PI capacity of the six non-recovered heifers started to increase within 1-2 days after the bacterial challenge. The maximum  $\alpha_1$ -PI response was reached in three days, being less than 2-fold. The  $\alpha_1$ -PI values remained increased for 1-2 weeks after the challenge. The other four animals showed no clear  $\alpha_1$ -PI response.

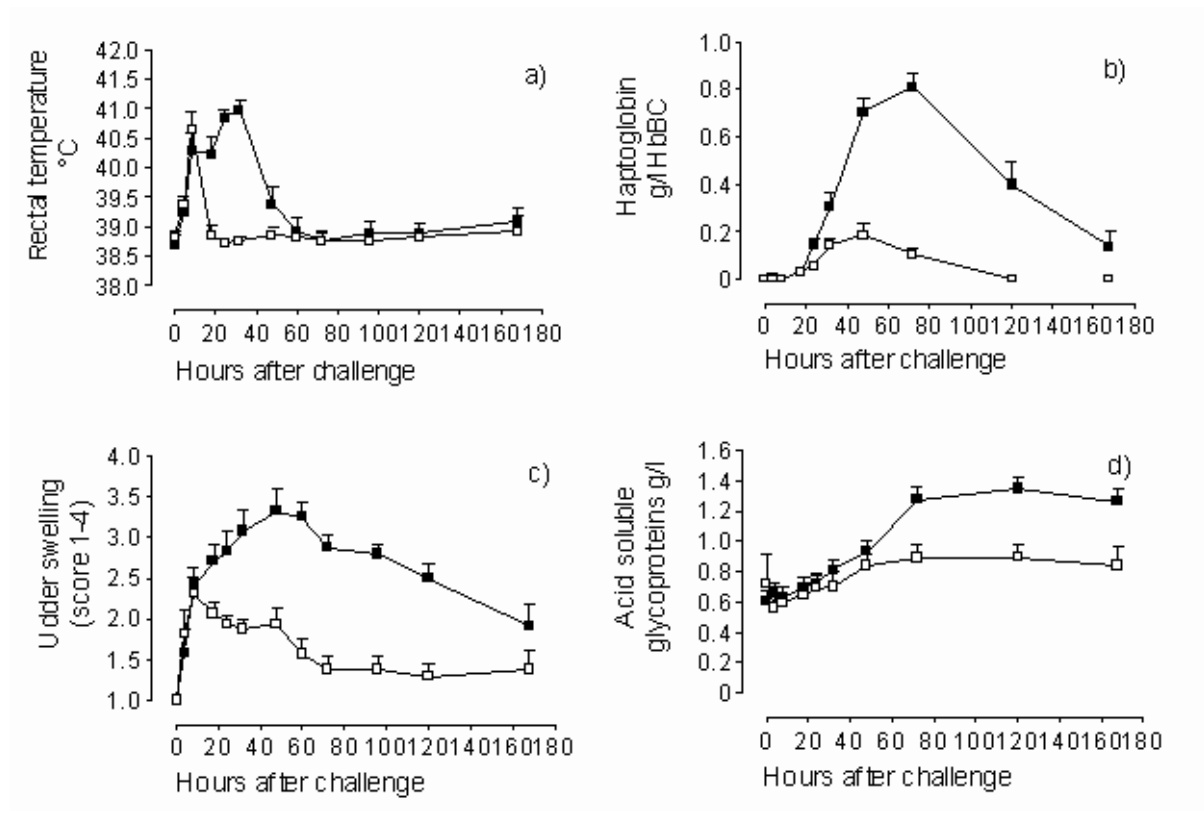


Figure 3. Mean ( $\pm$ SEM) a) rectal temperature, b) plasma Hp measured as g/l HbBC, c) udder swelling, and d) plasma acid soluble glycoprotein concentration in the four recovered heifers ( $\square$ ) and in the six non-recovered heifers ( $\blacksquare$ ) after intramammary challenge with *A. pyogenes*, *F. necrophorum* and *P. indolicus*.

## Dairy cows with experimentally induced *E. coli* mastitis (IV)

### Clinical and bacteriological results

The experimental infection with *E. coli* produced clinical mastitis in all six cows. The clinical response varied greatly, but the response of each cow followed the same pattern after both challenges. Three cows showed mild, and the other three either moderate or severe clinical signs of mastitis. Four cows recovered completely. Two most severely

affected cows became recumbent two days after bacterial challenge, and were later euthanized. The body temperature did not differ statistically significantly between cows. The mean decrease in daily milk production one day after challenge for mildly, moderately, and severely affected cows was 8, 35 and 77 percent, respectively. In the mildly and moderately affected animals, milk production normalized rapidly, within 1-2 days (Figure 4a). The *E. coli* numbers of the challenged quarters were higher in the moderately and severely affected cows than in the mildly affected ones (Figure 4b).

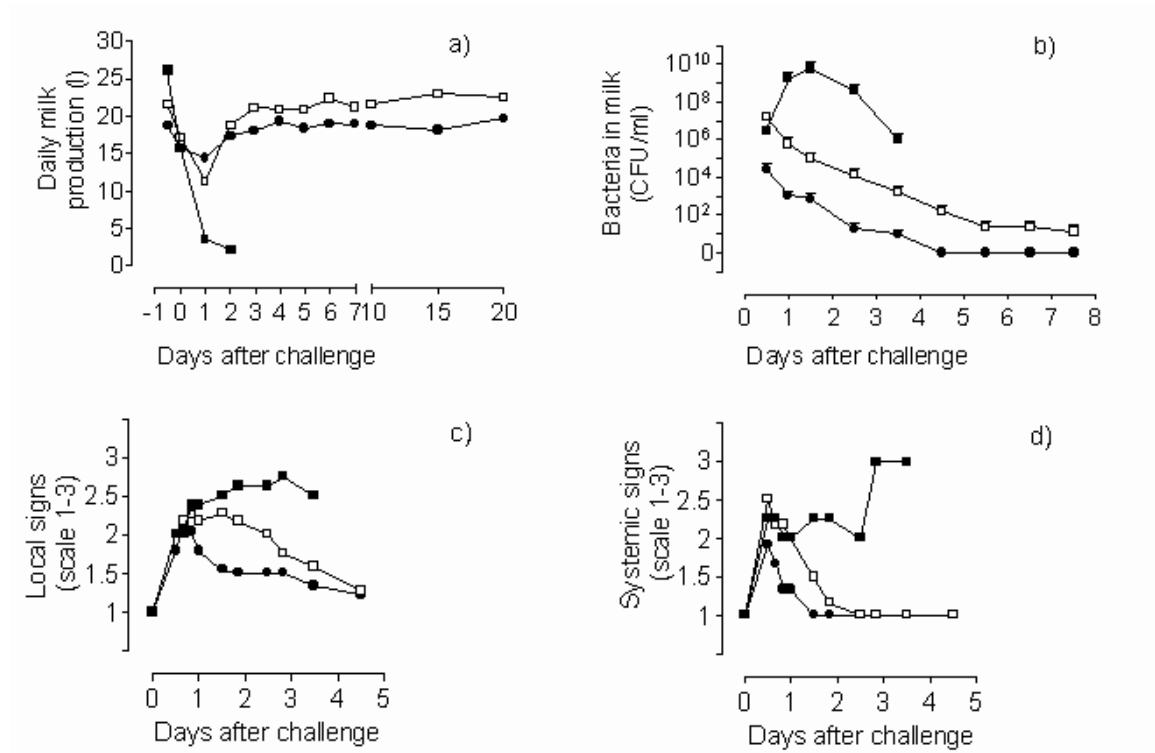


Figure 4. Mean ( $\pm$ SEM) a) daily milk yield, b) milk bacterial count, and score for c) local clinical signs and d) systemic clinical signs in the mildly (●), moderately (□), and severely (■) affected dairy cows after experimentally induced puerperal *E. coli* mastitis. The data are combined from two successive challenges carried out at three weeks intervals with six cows.

## APR

Bacterial challenge induced an increase in serum Hp concentration in all six cows. Hp levels were highly increased within 36 h, and the peak values were reached in 1-3 days

after challenge. Hp levels were normalized within one week. Hp response of the two cows with severe mastitis did not differ from that of the mildly or moderately affected ones (Figure 5a). Serum SAA increased in all six cows with experimental *E. coli* mastitis. The SAA response began uniformly. It subsided within 2 to 3 days, except in the severely affected animals, where the SAA values showed a continuous increase, until the animals were removed from the trial (Figure 5b).

Serum cortisol concentration showed a 10-fold increase at 12 hours after bacterial challenge in all experimental cows. After that, the cortisol levels decreased rapidly and reached the normal levels within 24-36 hours. The two cows with severe mastitis showed continuously elevated cortisol levels (Figure 5c). The mildly and moderately affected cows did not show any serum TNF $\alpha$  response during the experiment, while the two cows with severe mastitis showed a 2-fold response within two days after bacterial challenge, followed by return to background levels within three days after challenge (Figure 5d). Serum NO<sub>2</sub>/NO<sub>3</sub> concentration was decreased in all cows at 12 hours after bacterial challenge, followed by a slight increase in the severely affected cows at 1-2 days after challenge.

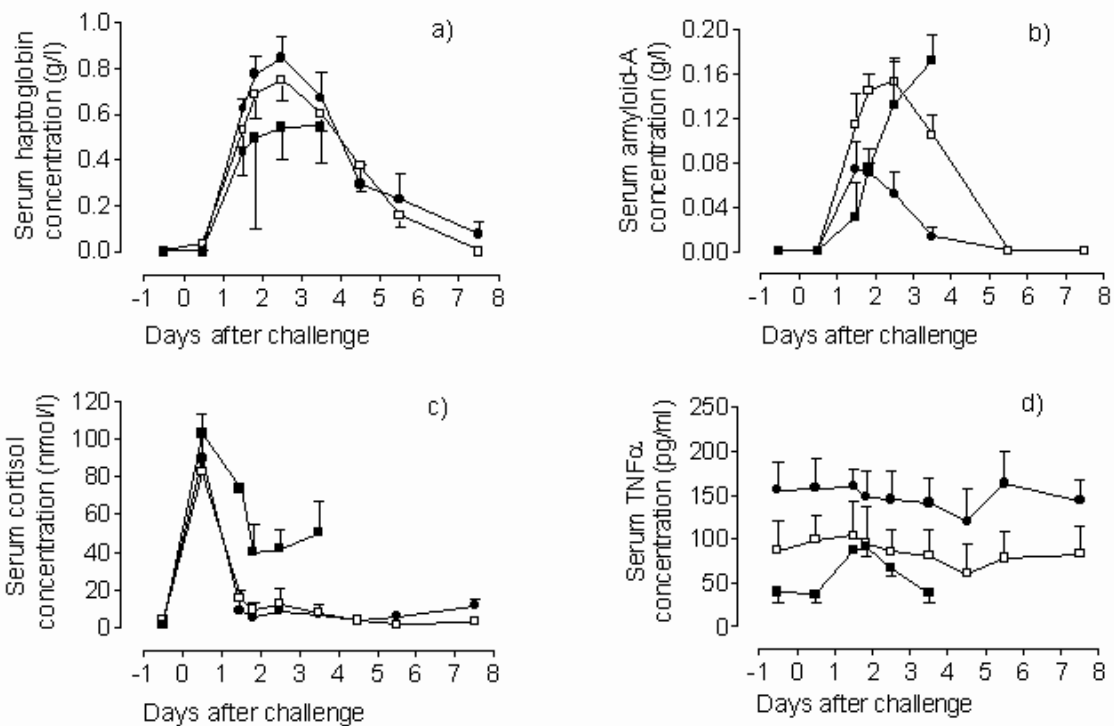


Figure 5. Mean ( $\pm$ SEM a) serum Hp, b) amyloid-A, c) cortisol and d) TNF $\alpha$  concentration in the mildly (●), moderately (□), and severely (■) affected dairy cows after experimentally induced puerperal *E. coli* mastitis. The data are combined from two successive challenges carried out at three weeks intervals with six cows.

## Dairy cows with acute postpartum metritis (V)

### Clinical results

The 19 cows with acute postpartum metritis showed putrid vaginal discharge which later changed to purulent or mucopurulent (Table 5). Five cows suffered from retained placenta (RP). Eight of them developed systemic clinical signs, fever and poor appetite, during the acute phase of the infection. The three most severely affected cows developed perimetritis with adhesions detectable in rectal palpation. The 10 control cows had no signs of acute uterine infection. The duration of acyclicity was twice as long as in the control group (30 versus 15 days) in the cows with acute postpartum metritis, and they also conceived poorly when inseminated. The three most severely affected cows were culled because of poor body condition and low fertility due to perimetrial adhesions.

Table 5. Quality of cervical discharge in the 19 cows with acute postpartum metritis.

Time after parturition	Putrid discharge		Purulent discharge		Cloudy mucopurulent discharge		No or clear discharge	
	%	n	%	n	%	n	%	n
4-11 days	100	19/19	0	0/19	0	0/19	0	0/19
15-22 days	0	0/19	58	11/19	32	6/19	11	2/19
32-44 days	0	0/18	6	1/8	28	5/18	67	12/18
41-50 days	0	0/17	0	0/17	12	2/17	88	15/17

### APR

Plasma Hp concentration remained low to moderate in most cows with acute postpartum metritis. Only the three most severely affected cows with perimetrial adhesions showed a strong Hp response. Nine cows with secondary mastitis also developed a Hp response. The 10 control cows had only constitutive plasma Hp concentrations, except four cows with acute clinical mastitis.

Alpha<sub>1</sub>-AG concentration was increased in 12 of 19 cows with acute postpartum metritis. On the day of diagnosis, three cows exhibited highly increased  $\alpha_1$ -AG levels. Two of them had dystocia. Two of them suffered also from RP (one cow had both dystocia and RP). During the first week postpartum, the 5 cows with RP had significantly higher  $\alpha_1$ -AG levels ( $P<0.05$ ) than the cows without RP. In the 10 control cows,  $\alpha_1$ -AG levels remained normal except for one animal with moderately increased  $\alpha_1$ -AG. During the first week postpartum, the difference in  $\alpha_1$ -AG levels between the cows with acute postpartum metritis and the controls was statistically significant ( $P<0.01$ ).



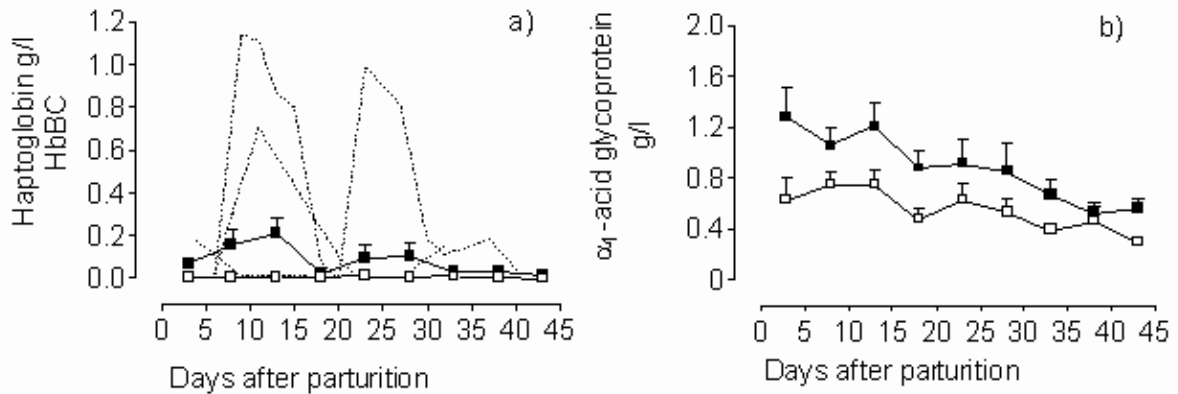


Figure 6. a) Mean ( $\pm$ SEM) plasma Hp measured as g/l HbBC, and b)  $\alpha_1$ -AG concentration of the 14 cows  $\alpha_1$  for Hp) with acute postpartum metritis (■) and the 6 puerperal cows which served as controls (□). The strongly divergent Hp profiles of three cows are shown separately with broken lines.

## Dairy cows with surgically-treated abdominal disorders (II)

### Clinical results

A total of 97 dairy cows had abdominal surgery. After surgery, 87 of them were returned to the herd. Four cows suffering from severe gastrointestinal disorders died or were euthanized after the surgery. A further 6 cows did not recover well and were culled.

### Haematology

Before surgery, the mean WBC counts were within normal limits in all diagnostic groups, but the mean blood neutrophil:lymphocyte ratios were rather high. The cows with dystocia showed the highest mean WBC and RBC count, PCV, and Hb concentration. The cows with traumatic reticuloperitonitis showed significantly higher plasma total protein and globulin levels than the other cows ( $P < 0.001$ ), while their RBC, RBC and Hb values were significantly lower ( $P < 0.001$ ) than those for the other cows. The cows with dystocia had significantly higher plasma albumin values and albumin:globulin ratios, and lower globulin values ( $P < 0.05$ ) than the others. After surgery, the above mentioned values began to normalize, except the high plasma total protein and globulin levels of the cows with traumatic reticuloperitonitis.

Table 6. Preoperative mean plasma values  $\pm$ SEM for selected haematological parameters of four diagnostic groups in the 97 surgically treated dairy cows. Statistical differences between one diagnostic group versus the others are marked as follows: \* $P < 0.05$  and \*\* $P < 0.001$ .

Parameter	Unit	Traumatic reticulo-peritonitis	Abomasal displacement or volvulus	Caesarean section	Explorative laparotomy	Reference value
		n=11	n=67	n=9	n=10	
Hemoglobin	g/l	104 $\pm$ 4 ***	127 $\pm$ 2	140 $\pm$ 7	133 $\pm$ 5	94-136
WBC	10 <sup>3</sup> / $\mu$ l	8.8 $\pm$ 0.7	6.9 $\pm$ 0.3	11.7 $\pm$ 2.5	8.7 $\pm$ 1.0	4-12
Albumin	g/l	35 $\pm$ 1	36 $\pm$ 1	40 $\pm$ 2 *	37 $\pm$ 1	30-36
Globulin	g/l	55 $\pm$ 2 ***	42 $\pm$ 1	35 $\pm$ 3 *	43 $\pm$ 2	30-35
Fibrinogen	g/l	11.6 $\pm$ 1.0 ***	6.2 $\pm$ 0.2	6.3 $\pm$ 0.5	6.2 $\pm$ 0.5	3-7
Haptoglobin	g/l HbBC	1.07 $\pm$ 0.09 ***	0.15 $\pm$ 0.03	0.07 $\pm$ 0.04	0.14 $\pm$ 0.08	0-0.05

### APR

Before surgery, plasma Hp levels for cows with traumatic reticuloperitonitis were significantly higher ( $P < 0.001$ ) than those in the non-infectious abdominal disorders showing either negligible or only mild Hp response. All cows with traumatic reticuloperitonitis had preoperative Hp values  $> 0.55$  g/l, while approximately 90% of the other cows had lower Hp values. In 51% of the other cows Hp was not detectable preoperatively by the photometric method. The effect of abdominal surgery on plasma Hp is illustrated in Figure 7.

Before surgery, also plasma Fb values for cows with traumatic reticuloperitonitis were significantly higher ( $P < 0.001$ ) than those for the other cows. Ten out of 11 cows with traumatic reticuloperitonitis had Fb values  $> 7$  g/l, whereas approximately 70% of the other cows had Fb values below that limit. The effect of abdominal surgery on plasma Fb is illustrated in Figure 7.

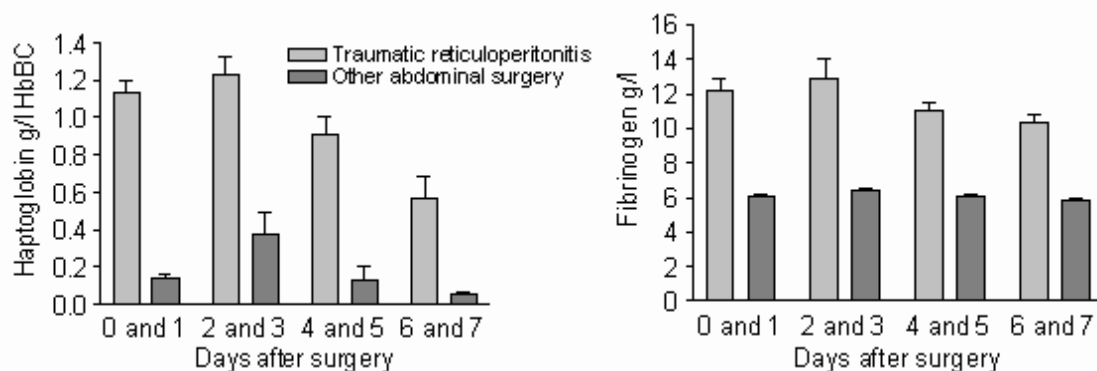


Figure 7. The effect of abdominal surgery on the mean ( $\pm$ SEM) plasma Fb and Hp concentrations in 11 cows with traumatic reticuloperitonitis and 86 cows with other gastrointestinal disorders or dystocia. The day 0 sample was taken preoperatively.

The secondary diseases, such as mastitis, ketosis, laminitis, and endometritis did not affect any of the monitored parameters significantly, although an increase in Hp values was occasionally seen with them. Mild wound swelling rarely caused any significant increase in APP levels. Neither haematological variables nor Hp and Fb were of value in predicting the outcome of the abdominal disorders.

#### ROC analysis

In traumatic reticuloperitonitis, many haematological parameters showed significant changes, and their diagnostic accuracy was evaluated using ROC analysis (Vida 1993, 1995; Zweig & Campbell 1993). ROC analysis plots a curve of *sensitivity* (true positive rate) versus *1-specificity* (true negative rate) for all possible threshold values of a parameter to be evaluated. The area under the curve (AUC) indicates the diagnostic accuracy of the examined parameter (Figure 8). To our knowledge, only one other study (Horadagoda *et al.* 1998) has used this approach to estimate the reliability of APP in differentiating inflammatory conditions in cattle. In our study, the cows with traumatic reticuloperitonitis were the positive diagnostic group, and the other non-infectious gastrointestinal diseases served as the negative control group. According to the AUC results, Hp proved to be the most accurate parameter for differentiating traumatic reticuloperitonitis from other abdominal disorders. Plasma Fb and globulin concentration were also effective in this respect.

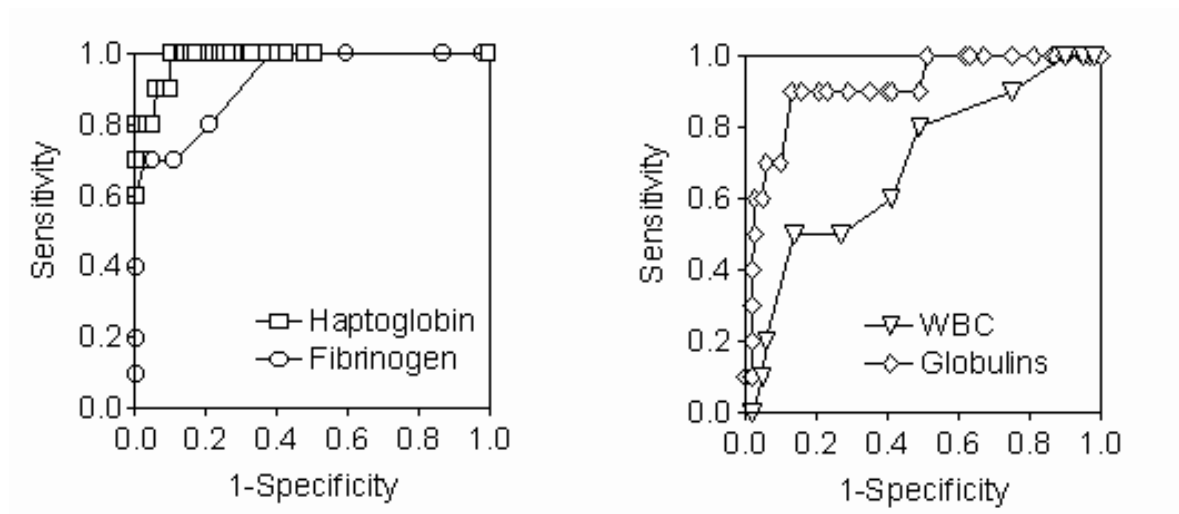


Figure 8. Non-parametric receiver operating characteristics (ROC) analysis of a) plasma Fb, b) plasma Hp, c) plasma globulin concentration, and d) white blood cell count in differential diagnosis of traumatic reticuloperitonitis (n=10) versus other gastrointestinal disorders (n=63) in dairy cows. Points of each curve represent the sensitivity and specificity of all possible threshold values for that parameter.

### Emergency slaughtered dairy cows (III)

#### *Meat inspection results*

Eighty emergency slaughtered Ayshire or Friesian dairy cows were divided into two groups according to their case history. Seventy-two cows suffered from diseases of infective, metabolic, or traumatic origin. Eight animals had only minor trauma and served as controls.

#### *APR*

Serum Hp concentration was increased in all diagnostic groups except controls, and the response was relatively uniform. Alpha<sub>1</sub>-AG response was more heterogenic. The highest alpha<sub>1</sub>-AG levels were recorded in cows with arthritis and mastitis, which was often chronic and purulent. Cows with generalized infections showed the highest gamma-globulin levels; high levels were also noted in cows with arthritis. Statistical comparison between the diseased and the control group showed that the diseased cows had significantly higher ( $P < 0.001$ ) Hp, alpha<sub>1</sub>-AG, and gamma-globulin levels when being culled (Table 7).

Table 7. Mean values (SEM for selected haematological parameters and the significance of difference in eight cows with minor injuries, which served as internal controls, and in the other 72 emergency slaughtered dairy cows.

Parameter	Unit	Minor injury (controls) n=8	Other cows (diseased) n=72	Significance of difference
Serum total protein	g/l	72.1 ± 4.1	76.8 ± 1.7	not significant
Gammaglobulins	g/l	14.3 ± 1.3	21.8 ± 1.4	<i>P</i> <0.001
Haptoglobin	g/l	0.01 ± 0.01	0.67 ± 0.07	<i>P</i> <0.001
α <sub>1</sub> -acid glycoprotein	g/l	0.47 ± 0.07	0.96 ± 0.06	<i>P</i> <0.001
Condemned meat/carcase	%	0 ± 0	21.2 ± 4.1	<i>P</i> <0.001

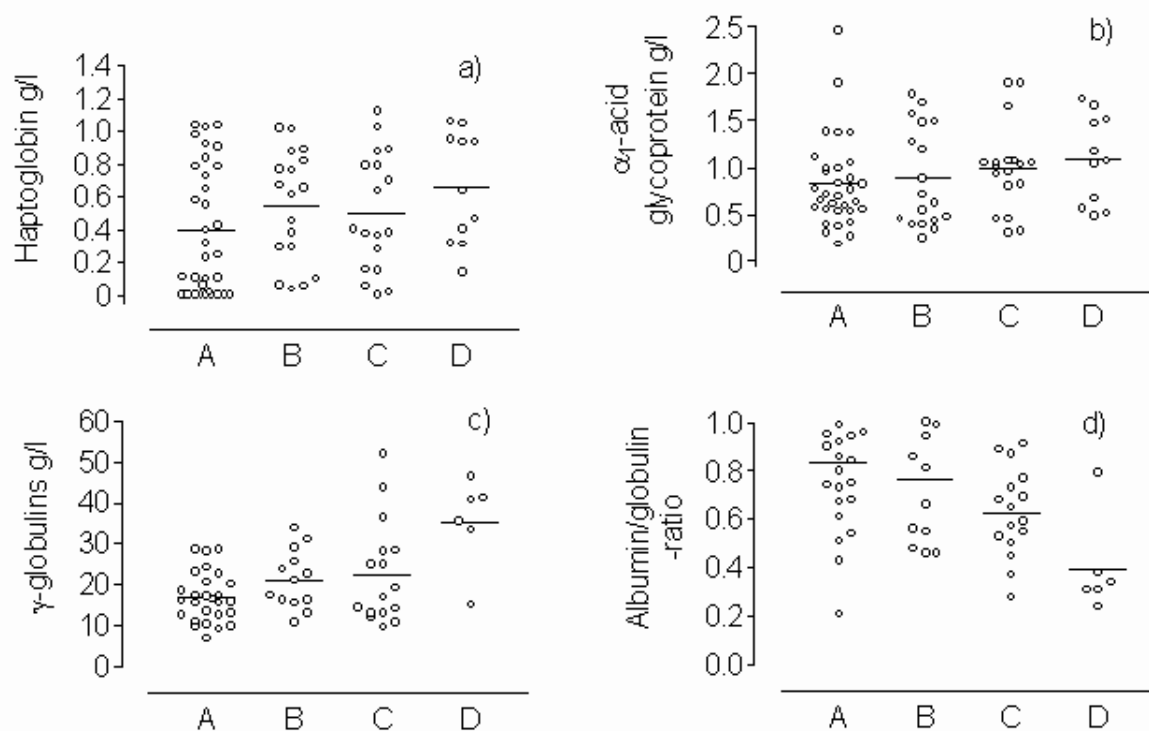


Figure 9. Scatterplots (with mean) for Hp, α<sub>1</sub>-AG and γ-globulin concentrations, and albumin:globulin-ratios in serum samples of four groups (A-D) of 80 emergency slaughtered dairy cows, classified according to percent amount of condemned meat per carcass. Note the different scale for each variable. A=0%, B=between 1 and 10%, C= between 11 and 40%, and D=100% of the weight of carcass condemned. There were no carcasses in which between 40% and 100% of their weight had been condemned.

#### Comparison of APP with the meat inspection results

Figure 9 illustrates the serum Hp, α<sub>1</sub>-AG and γ-globulin concentrations for individual cows in respect of the meat inspection results. Serum Hp and α<sub>1</sub>-AG concentrations were not related to the amount of condemned meat, while serum γ-globulin concentration was

significantly increased in totally condemned carcasses ( $P < 0.05$ ). In order to study the APR in muscle traumas, the 30 cows with muscle or minor traumas were examined separately. Alpha<sub>1</sub>-AG correlated more positively with the degree of muscle trauma than did Hp. The Pearson's correlation coefficient between serum Hp and  $\alpha_1$ -AG concentrations, and the percentage amount of condemned meat per carcass in 30 cows with muscle traumas or minor traumas were 0.19 and 0.54, respectively. The respective correlation coefficients for the activity of enzymes ASAT and CK were 0.62 and 0.63.

## DISCUSSION

### APR in dairy heifers and cows with mastitis

#### *Experimental mastitis models*

The two experimental mastitis models used here were very different by nature. The dairy heifers were challenged with facultative and obligatory gram-positive anaerobic bacterial strains which are able to produce purulent infections in cattle (Vecht *et al.* 1987). This mastitis form, often called as summer mastitis, occurs typically in heifers and dairy cows during dry periods. This may allow long development and the chronic character of the disease. In spontaneous cases, a diseased udder quarter is usually lost for milk production. Instead, the newly calved dairy cows were challenged with a gram-negative *E. coli* strain which is considered to be a pathogen which usually cannot persist in the mammary gland (Eberhart *et al.* 1979). The host animal is usually able to limit *E. coli* invasions and chronic infections are quite rare. Furthermore, pus formation is not characteristic to coliform infection, and coliform mastitis is uncommon during the dry period. The basic mechanism, how these bacteria induce APR, also differ. *E. coli* lipopolysaccharide is introduced to tissue macrophages largely via lipopolysaccharide-binding protein, which is an APP (Horadagoda *et al.* 1995, Bochsler *et al.* 1996), and CD14-receptor (Olson *et al.* 1995, Paape *et al.* 1996). An additional pathway that is CD14-independent and requires a functional *Lps* gene has lately been reported for mice (Haziot *et al.* 1998). According to the author's knowledge, no such specific mechanisms for the recognition of the above mentioned anaerobic bacterial strains have been described.

#### *Experimental anaerobic mastitis*

The experimental anaerobic mastitis model was used to study the bovine APR in purulent bacterial diseases caused by gram-positive bacterial strains, which are rather common in cattle. According to the APP measured, especially Hp, the model produced a strong APR which correlated positively with the severity and outcome of the disease.

The APP responses were in good agreement to those reported in calves after turpentine administration (Conner *et al.* 1988), and infection with *P. haemolytica* (Conner *et al.*

1989). A positive correlation between Hp/ASG levels and severity of the disease was seen in studies of Conner *et al.* (1988), who found that serum levels of Hp and  $\alpha_1$ -AG varied with the dose of turpentine administered subcutaneously in calves, whereas  $\alpha_1$ -PI levels did not. Alpha<sub>1</sub>-PI and Fb are constitutively secreted plasma proteins that can increase only a little above their relatively high baselines. These proteins also have a relatively long half-life. Instead, bovine Hp has a very low constitutive level, a high relative increase, and shorter half-life (Salonen *et al.* 1996), which makes bovine Hp a sensitive inflammatory marker. The experimental summer mastitis model suggests that bovine Hp has prognostic value in purulent bacterial diseases of dairy cattle: the recovered heifers produced only a moderate and temporary Hp response, in contrast to the quantitatively high and continuous response of the non-recovered heifers. It must be noted that also the clinical signs were effective indicators for the course of disease, especially the daily measurement of rectal temperature and the swelling of the udder.

#### *Experimental E. coli mastitis*

An experimental *E. coli* mastitis model was used to assess the bovine APR in gram-negative bacterial diseases. Of these, coliform mastitis is a common disease occurring worldwide. We previously used the same model in a treatment trial (Pyörälä *et al.* 1994). In the present study, postpartum cows were used because of their higher susceptibility to coliform mastitis. In contrast to our previous study (Pyörälä *et al.* 1994), the host response of the individual animals varied greatly: the severity of the disease varied from mild to fatal.

Experimental *E. coli* mastitis was able to trigger a strong serum Hp response, as we had also found previously (Salonen *et al.* 1996). Unlike the anaerobic mastitis model, the Hp response did not correlate positively with the severity of the disease, and Hp failed to discriminate between cows with a fatal outcome and the others. This may reflect the different infective agents involved: chronic purulent infections can probably induce a continuous Hp production, whereas short-lived coliform infections may only trigger a temporary Hp response. In contrast to Hp, SAA of the fatally infected cows rose progressively, signalling the continuation of the inflammatory process and deterioration of



the clinical condition of the cows. Thus, SAA proved to be a good quantitative marker for the severity of the disease. The different response patterns of Hp and SAA are presumably due to different induction pathways: pro-inflammatory cytokines can induce their synthesis in bovine hepatocytes in a different manner (Alsemgeest *et al.* 1996). Also their kinetics presumably differ and affect their profiles. However, the knowledge of APR and behaviour of APP in endotoxin shock with cardiopulmonary dysfunction and multi-organ failure is too limited to draw any strong conclusions. In contrast to anaerobic mastitis, rectal temperature did not play a significant role for assessing disease severity and outcome.

Comparison of the Hp results from the two experimental mastitis trials is somewhat difficult as the analysis of Hp was carried out using two methods. The two analytical methods were needed, because the more sensitive HPLC method could only be used with serum samples; plasma samples containing fibrinogen could not be eluted through the chromatographic column. The principle of both methods is the same: the Hp-HbCN complex is detected photometrically. However, there are noticeable differences between the methods. First, the serum samples for HPLC analysis were depolymerized with 2-mercaptoethanol, which affects the numeric results. Secondly, results from the two assays were expressed differently. The correlation between these two methods is good (Salonen *et al.* 1996), even though the numeric values are difficult to compare.

All cows responded to the intramammary challenge of *E. coli* with increased serum cortisol concentration. The continuing cortisol response in the two severely affected cows presumably reflected the continuation of bacterial growth and consequent APR in these animals; cortisol is needed for the down-regulation of APR (Baumann & Gauldie 1994).

TNF $\alpha$  plays a role in the pathogenesis of acute *E. coli* mastitis: bovine mammary macrophages are known to secrete TNF $\alpha$  in response to endotoxin (Pighetti & Sordillo 1994, Sordillo *et al.* 1995), and serum TNF $\alpha$  concentration has been reported to increase in naturally occurring coliform mastitis (Nakajima *et al.* 1997). In addition, Sordillo & Peel (1992) reported that TNF concentrations would be closely correlated with the severity of clinical disease. Here, the transient increase of serum TNF $\alpha$  concentration in the severely affected cows may reflect excess TNF $\alpha$  production and release. However, serum TNF $\alpha$

concentration did not prove to have a strong prognostic value in acute *E. coli* mastitis. Circulating TNF $\alpha$  levels may not be easy to interpret: Sordillo *et al.* (1991) reported elevated TNF levels in cows that died from endotoxemia, but Peel *et al.* (1990) failed to demonstrate circulating TNF during septicemic salmonellosis in calves.

Nitric oxide (NO) has been suggested to play a role in the development of septic shock by causing unresponsive vasodilatation and hypovolemia (Payen *et al.*, 1996). The increase in serum NO<sub>2</sub>/NO<sub>3</sub> concentration, the two metabolites of NO, in the severely affected cows may have indicated excess nitric oxide (NO) production, because the feed intake and the consequent intake of exogenous NO<sub>2</sub>/NO<sub>3</sub> were reduced due to the disease. Nevertheless, it was concluded that serum NO<sub>2</sub>/NO<sub>3</sub> was not a reliable marker for indigenous NO production during acute coliform mastitis in the bovine.

The clinical condition of the two severely affected cows started to deteriorate at the 2<sup>nd</sup> day after bacterial challenge, which was about 36 hours from the onset of clinical signs. This makes it difficult for the practicing veterinarian to find the animals with poor prognosis for more intensive treatment early enough. The severity of mastitis was significantly related to bacterial counts in milk. Our findings support those from many previous studies (Hill *et al.* 1979, Lohuis *et al.* 1990, Katholm & Andersen 1992, Vandeputte-van Messom *et al.* 1993, Shuster *et al.* 1996). There was also a pronounced decrease in milk production in the two cows with fatal mastitis. Milk production one day after challenge proved to be an accurate indicator for development of severe coliform mastitis, which has been seen also in previous reports (Lohuis *et al.* 1990, Vandeputte-van Messom *et al.* 1993). Milk bacterial count and drop in the milk yield together seem to have a good predictive capacity for the prognosis of acute coliform mastitis. According to Monfardini *et al.* (1999), the first 10 hours after challenge appear to be critical for the development and the severity of *E. coli* mastitis. For most inducible APP the time lag is too short to achieve an informative serum response. In other words, they are not yet very informative at the time clinical signs appear.

### *APR in individual animals*

As the both experimental mastitis models demonstrated, the susceptibility of individual heifers/cows to experimental mastitis varied considerably, and played the key role in the outcome of the disease. This information would be difficult to obtain from clinical field studies, where all the factors involved, such as the infective organism, the host animal, and the environment together represent too much variation for studying the effect of host response objectively. Under field conditions the time lag from the onset of the infection to the examination time is not known, which is problematic for a study of APR.

In order to improve the host's resistance to infectious diseases we must acquire better knowledge about the interactions between infective agents and the host, and the nature of both innate non-specific and adaptive specific immunity. Key factors are the role of APR in resisting new infections, the interaction between APR and the acquired immunity, and the variability of APR between individuals. Not only the genotype, but the age, stage of lactation, and the nutritional status play a role in the responsiveness of a dairy cow. For example, a decline in CRP has been reported to take place after feeding of energy deficient diet to dairy cows (Seidler *et al.*, 1998), and chromium supplementation to the diet reduced the APR, as indicated by serum Hp, in newly arrived feeder calves (Wright *et al.* 1995).

### **APR in dairy cows with metritis**

The most frequently isolated bacterial species in metritis were partly the same as those discussed previously: *A. pyogenes* and *E. coli* bacteria were found in almost all cows with acute postpartum metritis. Naturally, the severity of disease varied more in naturally occurring metritis than in experimental mastitis models. Fewer animals had systemic clinical signs, such as fever and inappetite. The Hp patterns were also different from those of acute clinical mastitis: only the most severely affected animals with perimetrial adhesions showed a high Hp response. The result that not all animals with systemic signs showed a Hp response was not expected. This result suggests that a bovine metritis does not necessarily include a hepatic Hp response, and that a high Hp response probably reflects a uterine inflammation which is not localized to endometrium only. Therefore, determination of Hp can be of value in indicating the severity of an acute uterine infection

and predicting the subsequent fertility of a postpartum dairy cow. This statement is supported by Bertoni *et al.* (1997), who reported that high Hp concentrations (>1 g/l) increased the risk of early culling and may impair reproductive functionality. Interestingly, it has been found that rats with endometriotic lesions synthesize and secrete a Hp-like protein; this finding may lead to better understanding of the pathophysiology of uterine disorders (Sharpe-Timms *et al.*, 1998).

The significantly increased  $\alpha_1$ -AG levels of the cows with acute postpartum metritis indicated the presence of APR. This suggests that an acute postpartum metritis affects the performance of a postpartum cow. In contrast to Hp, the  $\alpha_1$ -AG levels did not correlate with the severity of disease, and consequently the capacity of  $\alpha_1$ -AG in differentiating genital infections was poor. The  $\alpha_1$ -AG levels seemed to be more related to difficulties at calving. This suggests that parturient trauma may induce an  $\alpha_1$ -AG response. Alpha<sub>1</sub>-AG levels seemed also to be related to the presence of RP. Retained placenta often induces an endometritis with pus formation (Lowder 1993), and  $\alpha_1$ -AG response is known to take place in purulent diseases of cattle (Motoi *et al.* 1992).

#### **APR in surgically treated dairy cows**

Conventional haematology had no particular diagnostic value to assess the condition of surgically treated abdominal disorders. There were two groups of cows which had diverse haematological profiles. The cows with dystocia had mild haemoconcentration and high WBC counts before surgery; high WBC counts are typical in cows at parturition (Jain 1993). On the other hand, the cows with traumatic reticuloperitonitis had low RBC values; this is a common feature for persisting inflammatory and infectious conditions, such as traumatic reticuloperitonitis (Smith 1990). These animals had also exceptionally high total plasma protein and globulin levels due to chronic purulent disease.

The high preoperative Fb values in cows with traumatic reticuloperitonitis are well known. As reported by McSherry *et al.* (1970), high Fb values are a typical feature of several infectious diseases in cattle, such as peritonitis. In our study, surgical trauma did not prove to increase plasma Fb concentrations. This is in contrast to the results of Fisher *et al.* (1997), who found a significant increase in plasma Fb (>10 g/l) after surgical castration of

bull calves. This controversy can be due to different age of the animals and methodological differences: our semiquantitative heat precipitation technique is rather different from that of Fisher *et al.* (1997) using frozen plasma samples and a commercial biochemical assay kit for the analysis of Fb.

The cows with non-infectious abdominal disorders had relatively low preoperative plasma Hp levels, indicating the presence of either no APR or a mild one. Abdominal surgery induced a moderate Hp response peaking 2-3 days after surgery. The response was milder than after surgical castration of bull calves reported by Fisher *et al.* (1997), who found approximately 10-fold increase of Hp. The result indicates that surgical castration of bull calves with an open method induces a stronger APR than paralumbar fossa laparotomy of dairy cows. In addition, the cows with abdominal disorders often had a minor APR before surgery, which partly explains the only moderate postsurgical Hp response. Morimatsu *et al.* (1992) reported more than 50-fold increase of serum Hp after rumenotomy using a single radial immunodiffusion assay method. The high relative increase was affected by the reduction of polymeric Hp to a homogenous form with cysteine which markedly increased the sensitivity of the assay remarkably. The only moderate postsurgical APR indicates that abdominal surgery does not affect the dairy cow very strongly. A possible explanation for this would be that the net amount of traumatic tissue in abdominal surgery is usually rather small, thus leading to only minor or moderate APR. Clinical observations support this: dairy cows with surgically treated abomasal disorders often recover surprisingly well, without any significant loss of weight or milk yield. The high Hp levels of the cows with traumatic reticuloperitonitis are in line with Makimura & Suzuki (1982), who found high Hp concentrations in cows with traumatic reticulitis and pericarditis. In a recent study, SAA and Hp were found to be potentially useful to discriminate between acute and chronic inflammatory conditions (Horadagoda *et al.* 1998). In that study, using the ROC method, SAA was found to be the most reliable acute phase protein in differentiating cattle with acute and chronic inflammation.

Shakespeare *et al.* (1989) observed considerable differences between the postoperative APP patterns in human patients with infectious complications and in patients with normal recovery from surgery. In dairy cows, Fb would be of value in detecting secondary

infectious complications after abdominal surgery, because it does not seem to respond markedly to the surgery itself. It must be noted that plasma Fb concentrations can be decreased by local consumption in the wound and abdominal area. There are commercial tests on the market to detect degradation products of fibrin and fibrinogen, which have been evaluated in dogs and horses (Stokol *et al.* 1999, Sandholm *et al.* 1995), but these tests have not been applied to cattle. Also Hp could be used for the follow-up of infectious complications, but the preoperative levels and the moderate response to surgical intervention should be taken into account. Hp concentrations can also be affected by a loss due to haemolysis or by a decreased synthesis due to hepatic insufficiency (Turner 1995). Here, the complications were mainly related to the gastrointestinal function, and did not trigger a significant APR.

According to the results from the ROC analysis, Hp was the most accurate parameter for detecting traumatic reticuloperitonitis. This is in agreement with Alsemgeest *et al.* (1994b), who found Hp effective for detecting serious inflammatory diseases in cows, and Skinner & Roberts (1994), who reported blood Hp to be a more efficient indicator of bacterial infection than routine haematological variables. Fb was also effective in this respect. The difference between Hp and Fb was mainly the increased sensitivity of Hp. Blood globulin concentration proved also to be an accurate marker for traumatic reticuloperitonitis. Increased globulin levels are characteristic to chronic inflammation where large amounts of immunoglobulins are produced (Cole *et al.* 1997). Instead, the diagnostic capacity of total WBC count and neutrophil-lymphocyte-ratio were poor. This was expected, because many chronic diseases in cattle are associated with normal WBC counts (Kidd 1991, Cole *et al.* 1997).

### **Time-profile of APR**

In articles I, II, IV, and V bovine APR was examined horizontally, including both sequential clinical examinations and plasma/serum APP determinations. This horizontal approach reveals a major problem affecting the interpretation of APP analyses in clinical diagnostics: in clinical cases the time of initiation of an inflammatory process is not known. This makes it difficult to interpret the results of APP analyses, because the APP concentrations are time-dependant. Depending on the APP it usually takes 1-3 days to

obtain a significant response. On the other hand, APP profiles may not indicate the cessation of APR early enough. To avoid these problems, the accuracy of clinical diagnostics can be improved by a simultaneous evaluation of several diagnostic variables, both clinical and laboratory parameters. The variables should have different time-profiles during an APR, including both rapid and slowly responding markers, to achieve a high diagnostic accuracy (Thompson *et al.* 1992, Gruys *et al.* 199?).

### **APR in emergency slaughtered dairy cows**

Most of the emergency slaughtered animals showed APR when being culled. The reason is that there is usually a delay of a few days after the onset of clinical disease before the cow is slaughtered, which is long enough for the development of APR detectable by APP. Transportation did not seem to trigger any APR detectable by Hp or  $\alpha_1$ -AG. As APR has several metabolic effects on the host animal, it may influence meat quality of the diseased animals.

Serum Hp and  $\alpha_1$ -AG could be used to accurately differentiate between the diseased animals and those with normal clinical condition. This supports the previous findings that APP can be used for separating diseased animals from the normal slaughterhouse material (Saini & Webert 1991, Gruys *et al.* 1994). Gruys *et al.* (1993) also were able to demonstrate good correlation between APP and the severity of pathological lesions. The association between pathological lesions at slaughter and increased Hp levels has also been reported by Young *et al.* (1996). Hp in particular has been considered as a decisive indicator for pathological lesions, because it is unmeasurable in normal cattle but elevated in the presence of most diseases (Saini *et al.* 1998). In our study, serum Hp and  $\alpha_1$ -AG were not quantitatively predictive of the meat inspection result. Predicting the amount of condemned meat is presumably not possible by means of APP. The fact that individuals with high APP levels can be accepted during the routine meat inspection process raises the question of reliability of the traditional meat inspection process. Diseased animals are not always detected and may cause occasional quality problems for the meat industry and eventually perhaps also for the consumers.

Serum  $\gamma$ -globulin concentration was predictive of the meat inspection result a little more effectively than Hp and  $\alpha_1$ -AG. The reason for this was that totally condemned carcasses had very high  $\gamma$ -globulin levels. Major components of the electrophoretic  $\gamma$ -globulin fraction are immunoglobulins, which respond slower than Hp and  $\alpha_1$ -AG (Jain 1993). This result suggests that cows with more chronic lesions are also more likely to be rejected. Gammaglobulinemia is a typical finding in chronic purulent diseases (Jain 1993) and due to macroscopic changes such cows are easily identified in meat inspection.

Muscle traumas induced a strong APR as indicated by serum Hp and  $\alpha_1$ -AG. This must be taken into consideration when interpreting APP results from recumbent cows. Downer cows often suffer from fatty liver, especially during the periparturient period, and serum Hp is known to increase in cattle with fatty liver (Yoshino *et al.* 1992). Alpha<sub>1</sub>-AG showed a relatively high positive correlation with the extent of muscle trauma, which suggests that muscle damage can induce the synthesis of bovine  $\alpha_1$ -AG. However, muscle specific enzymes are the most accurate means to estimate the extent of muscle trauma.

#### **APR in other diseases of dairy cattle**

Our study design did not include viral infections. One indication for introducing APP to veterinary clinical diagnostics would be their possible role in the differential diagnostics of bacterial and viral diseases, an indication which has not been evaluated thoroughly. In humans, Young *et al.* (1991) found no evidence that CRP, the APP of choice in clinical diagnostics in humans, could help in differentiating between bacterial and viral infections, although that has been suggested (Thompson *et al.* 1992). In Finland, viral infections of cattle to be evaluated would include respiratory diseases, such as bovine respiratory syncytial virus infections, and gastrointestinal diseases caused by different viruses, such as adeno-, corona- and rotaviruses. In addition, bovine APR could also be studied in different internal parasitic diseases, such as coccidiosis, ostertagiosis, and cryptosporidiosis.



## CONCLUSIONS

According to the APP profiles, APR played a role in all clinical conditions involved in this study. The intensity of APR varied between different conditions, being highest in bacterial diseases, particularly in purulent conditions. Typical diseases with high APR were mastitis, arthritis and traumatic reticuloperitonitis. Abdominal surgery induced only a minor to moderate APR without a marked effect on the host. Acute postpartum metritis produced a variable APR. Emergency slaughtered dairy cows usually had a moderately high APR.

APP proved to be accurate markers for various inflammatory conditions of dairy cows, the accuracy being better than that of clinical examination and conventional haematology. The determined APP fell into different categories according to their response patterns. Blood  $\alpha_1$ -AG,  $\alpha_1$ -PI and Fb produced a mild to moderate relative response, whereas blood Hp and SAA had a high relative response. Hp proved to be a good diagnostic and prognostic marker for purulent and more chronic infections, whereas SAA was a good marker in more acutely diseased animals. One major advantage of Hp and SAA over the clinical examination is the better quantification of the disease severity than obtained by clinical examination only. The tendency for larger dairy herds may increase the need for diagnostic APP tests, as less time may be spent on examination of individual animals.

In bacterial diseases, APP do not predict survival very efficiently, because animals with severe bacterial infections usually show high APP levels. Most accurate information is obtained by a repeated determination of a selected APP during the course of disease. APP do not directly reflect gastrointestinal function and, therefore, they are not effective predictors for recovery from abdominal disorders, except secondary bacterial complications. In the case of an acute uterine infection, high APP levels may reflect a severe infection which can reduce fertility. APP can also play a role in meat inspection. Indications for their use can be discrimination between healthy and diseased animals, and quantification of the inflammatory status of slaughter cattle.

The susceptibility of individual heifers and cows to experimental mastitis varied strongly. According to APP patterns, clinical symptoms, and bacterial recovery, the experimental

animals were clearly different to those with a mild and short-lived disease, and those with a more severe and persistent infection. In other words, individual factors play a key role in resisting bacterial infections in dairy cattle. This result indicates that we should remember the importance of host resistance in order to improve the disease control in dairy units. The pathophysiological factors that are involved in disease resistance should be studied in detail.

In this study, bovine APR was examined horizontally, including both sequential clinical examinations and plasma/serum APP determinations. This horizontal approach revealed a major problem affecting the interpretation of APP analyses in clinical diagnostics: in clinical cases the time-profile of an inflammatory process is not known. This makes it difficult to interpret the results of APP analyses, because the APP concentrations are time-dependant. To avoid this problem, the accuracy of clinical diagnostics can be improved by a simultaneous evaluation of several diagnostic variables having different time-profiles during an APR.

In bovine medicine, there is a need for several kinds of APP measurements. In dairy practice, cow-side tests would be optimal for clinical use to improve the accuracy of clinical diagnostics. Here, the methodology should be relatively easy, rapid and cost-effective, and preferably at least semi-quantitative. In the meat industry, rapid serial analytic systems would be needed for the quantification of APR. More advanced immunological and molecular techniques are needed in medical science, where highly accurate methods for the qualification and quantification of APR are required.

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