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# Acute Phase Response in Animals: A Review

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**The acute phase response is a complex systemic early-defense system activated by trauma, infection, stress, neoplasia, and inflammation. Although nonspecific, it serves as a core of the innate immune response involving physical and molecular barriers and responses that serve to prevent infection, clear potential pathogens, initiate inflammatory processes, and contribute to resolution and the healing process. Acute phase proteins, an integral part of the acute phase response, have been a focus of many applications in human diagnostic medicine and recently have been identified in common animal species. Potential applications to diagnosis, prognosis, assessment of animal health, and laboratory animal welfare are readily apparent.**

**Abbreviations:** APP, acute phase protein; APR, acute phase response; CRP, C reactive protein; SAA, serum amyloid A.

Acute phase proteins (APP) are blood proteins primarily synthesized by hepatocytes as part of the acute phase response (APR). The APR is part of the early-defense or innate immune system, which is triggered by different stimuli including trauma, infection, stress, neoplasia, and inflammation. The APR results in a complex systemic reaction with the goal of reestablishing homeostasis and promoting healing. The APR has been referred to as the 'molecular thermometer' whereby quantitation of individual APP can provide an assessment of the response to the triggering event.[10,82,93]

APP have been well recognized for their application to human diagnostic medicine and have been described to have value in the diagnosis and prognosis of cardiovascular disease, autoimmunity, organ transplant, and cancer treatment.[15,16,24,36,50,57,73,102] C-reactive protein (CRP) was the first APP described (in the early 1930s), and its presence early during pneumococcal infection of monkeys and humans thereby led to the coinage of 'acute phase' term.[1,123] Today, CRP remains an APP of primary interest in humans, where it is a major marker of infection, autoimmune disease, trauma, malignancy, and necrosis including myocardial infarction.[92] Furthermore, CRP has been proposed as a marker for wellness assessments, which is a common role proposed in many studies of human and animal APP.[73]

Although the study of serum proteins in animals was commonly conducted in research investigations in the mid-1900s, the wider application of APP in veterinary medicine was not reported until the early 1990s. Several review papers with descriptions of APP and APR in companion animals and rodents are available.[10,39,82,93] Like human APP, animal APP have been well documented to be sensitive to similar triggering events. In large animals, APP have been further proposed as markers of 'herd health.'[40,82,93] In companion animals, APP have especially been identified for prognostic applications.[10] The use of APP in laboratory animals has essentially been limited to research based investigations, but APP have been proposed as an objective marker for humane experimental endpoints.[39,89] The following sections summarize the biologic effects of the APR, current issues and options in the quantitation of APP and APR, applications of APP in the diagnosis and prognosis of disease in animal species, and proposed applications of APP in laboratory animals.

## The Acute Phase Response

Innate immunity fulfills an important role in the body's early defense mechanism and serves to initiate the acquired immune response. The innate immune system is very diverse and includes physical barriers, phagocytes, complement, and toll-like receptors which serve to prevent infection, eliminate potential pathogens, and initiate the inflammatory process.[64] Later aspects of induced innate responses are dependent on cytokines and chemokines, which are generated by activated cells including monocytes, macrophages, fibroblasts, endothelium, platelets, keratinocytes, and T cells.[64] These proinflammatory signals, including IL1, TNFα, and IL6, have numerous effects throughout the body including inducing the APR (Figure 1). The APR is a core part of the innate immune response and is observed across all animal species. In fact, counterparts of mammalian APP have been identified in invertebrates and fish, in which the APR is proposed to be more robust than that in mammals to compensate for a less-evolved adaptive immune response.[4,100]

The APR may result in changes in more than 200 proteins grouped as either positive APP or negative APP.[72] In nearly all animal species, albumin represents the major negative APP which, during the APR, decreases in blood concentration and may represent either selective loss of albumin due to renal or gastrointestinal changes or a decrease in hepatic synthesis.[72] The decrease in albumin synthesis is postulated to allow for the unused pool of amino acids to instead be used to generate positive APP and other important mediators of inflammation.[90] Positive APP are those that increase during the APR. They are further classified as major, moderate, or minor, depending on the magnitude of increase during the APR. Traditionally, major proteins represent those that increase 10- to 100-fold, moderate proteins represent those that increase 2- to 10-fold, and minor proteins represent those with only a slight increase.[10] These classifications may vary

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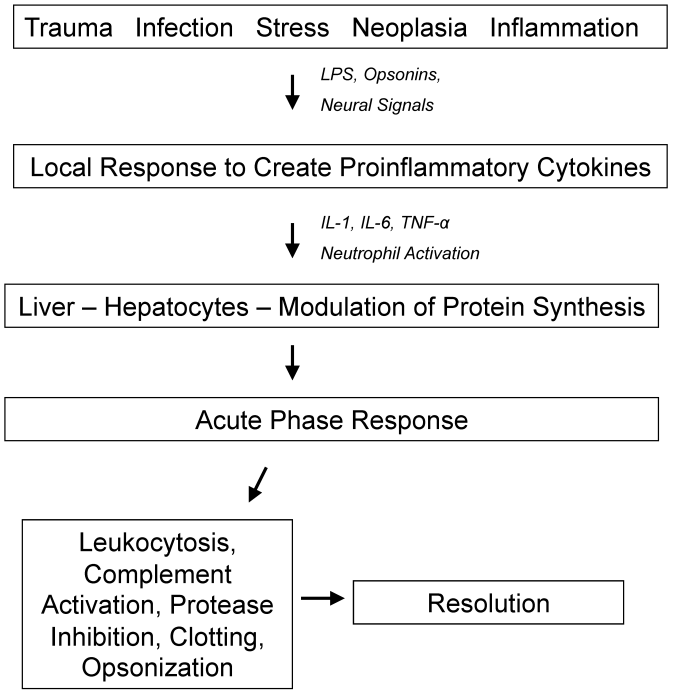


**Figure 1.** The acute phase response. The figure reflects information drawn from references 10, 82, and 93.

slightly within different APR publications, although the implied changes by category remain the same.[90] Major proteins often are observed to increase markedly within the first 48 h after the triggering event and often have a rapid decline due their very short halflife.[70] Moderate and minor proteins follow in magnitude of response and may both increase more slowly and be more prolonged in duration, depending on the status of the triggering event. Moderate and minor APP may be observed more often during chronic inflammatory processes.[10,93] Species differences are readily apparent in the classification of positive APP (Figure 2).

The biologic functions of APP are vast and have been reviewed.[10,70,82,93,116] A summary of these key observations follows. For example, CRP can act as an opsonin by binding residues and polysaccharides on bacteria, fungi, and parasites to activate complement and phagocytosis. In addition, CRP can both upregulate and downregulate cytokine production and chemotaxis. Serum amyloid P is thought to be an analog to CRP in some species. Serum amyloid A (SAA), another APP often classified as a major protein, has been demonstrated to result in chemotaxis of monocytes, polymorphonuclear cells, and T cells. In addition, SAA has marked inhibitory effects and is assumed to be important in the down regulation of the inflammatory process. Haptoglobin reduces oxidative damage associated with hemolysis by binding free hemoglobin. In addition, it has been observed to have bacteriostatic and immunomodulatory effects. The APP α1-acid glycoprotein binds LPS and inhibits its activity; α1-acid glycoprotein also can bind to numerous drugs and may sustain drug transport levels even with a decrease in albumin, the negative acute phase protein. Whereas α1-acid glycoprotein can also downregulate neutrophils and complement, α2-macroglobulin is 1 of several APP that have protease inhibitory activity and are important in removing enzymes released during injury. Major acute phase protein (porcine) (or pig-MAP) has been identified in porcine species and inhibits trypsin. Ceruloplasmin scavenges free radicals. Fibrinogen provides a substrate for fibrin formation and thus is important in tissue repair. Transferrin is proposed to be a positive APP in avian species, although in most mammals, it is considered a negative APP. In summary, positive APP all have multiple functions including modulating the immune system, protein transport, and tissue protection from damage by the inflammatory process.

## Assays of Acute Phase Proteins

In basic health assessments, total protein and albumin can be measured on is commonly measured on automated chemistry analyzers, and globulin is a value calculated from these measurements. In human and veterinary medicine, protein electrophoresis is a diagnostic tool that has widely been applied to the study of the APR for more than 40 y.[2,17,72,107] Although rarely solely diagnostic of a particular disease, protein electrophoresis is an excellent method for the detection of acute and chronic inflammatory processes and stimulation of humoral immunity. Veterinary applications include its ancillary use in the diagnosis of feline infectious peritonitis, ehrlichiosis, and myeloma and, more recently, in the diagnosis and prognosis of infectious diseases in avian species.[19,20,72]

The general technical principle of protein electrophoresis involves overlaying plasma or serum on a thin agarose gel. An electric current is applied to the gel, which causes the proteins to migrate according to their charge and size. The movement of the proteins creates bands in the gel, which can be quantitated by densitometry. Protein electrophoresis can be performed by using commercially available systems, which allow for on-demand performance, are relatively inexpensive, require only a few microliters of serum, and provide results within a few hours. In addition, multiple samples can be analyzed simultaneously, and overall changes in the APR can be quantitated. The proteins that are resolved by protein electrophoresis are albumin and the 4 fractions of globulins referred to as α1, α2, β, and γ. Each globulin fraction is composed of APP or immunoglobulins (or both). Protein electrophoresis provides a more accurate reflection of the albumin:globulin ratio, given that as albumin methodologies in chemistry analyzers are optimized for human albumin and that bromocresol green can bind animal globulins with extended reaction times.[41,115] Therefore, protein electrophoresis can provide more accurate albumin quantitation and visualization of globulin fractions to monitor the overall progression of the APR. Protein electrophoresis does not enable quantitation of single proteins but rather of groups of proteins that are mediators of acute inflammatory processes. More than 200 blood proteins have been defined and are likely present in protein electrophoresis fractions.[72] Changes in proteins with low concentrations may not be detected by this technique. Protein electrophoresis electrophoretograms from commercial systems are thought to reveal changes in approximately 30 major serum proteins. α1 globulins include α1-antitrypsin and α1-acid glycoprotein, α2 globulins include α2-macroglobulin and haptoglobin; β globulins include transferrin, SAA, fibrinogen, and CRP; and γ globulins primarily comprise IgG.[72] Although reference intervals are available for most common mammalian species, these ranges often are derived from nonlaboratory animals and thus should be evaluated critically before their application. We recently published normal reference intervals for common inbred strains of mice, including age-relative

| Species | Major (>10-fold increase) | Moderate (1- to 10-fold increase) |
|---|---|---|
| Cat | α1-acid glycoprotein, serum amyloid A | haptoglobin |
| Chicken | none | α1-acid glycoprotein, ceruloplasmin, serum amyloid A, transferrin |
| Cow | haptoglobin, serum amyloid A | α1-acid glycoprotein, C-reactive protein, fibrinogen |
| Dog | C-reactive protein, serum amyloid A | α1-acid glycoprotein, ceruloplasmin, haptoglobin |
| Goat | haptoglobin, serum amyloid A | fibrinogen |
| Horse | serum amyloid A | fibrinogen, haptoglobin |
| Human | C-reactive protein, serum amyloid A | α1-acid glycoprotein, fibrinogen, haptoglobin |
| Mouse | haptoglobin, serum amyloid A, serum amyloid P | C-reactive protein, fibrinogen |
| Nonhuman Primates | C-reactive protein | α2-macroglobulin, fibrinogen, serum amyloid A |
| Pig | haptoglobin, serum amyloid A, major acute phase protein | α1-acid glycoprotein |
| Rabbit | haptoglobin, serum amyloid A | α1-acid glycoprotein, C-reactive protein, fibrinogen |
| Rat | α1-acid glycoprotein, α2-macroglobulin | C-reactive protein, fibrinogen, haptoglobin |
| Sheep | haptoglobin, serum amyloid A | α1-acid glycoprotein, C-reactive protein |

**Figure 2.** Major and moderate acute phase proteins in different animal species. The figure reflects information drawn from references 10, 13, 39, 59, 82, 93, and 108.

reference intervals, by using a commercially available agarose-based electrophoresis system.[137] Investigators planning to implement protein electrophoresis or APP as tools in laboratory animal medicine should consider establishing a baseline for species and strains, given that the variation present in normal resting values likely is unknown.

Individual APP have been documented in many species by using ELISA, radioimmunoassay, nephelometry, and immunoturbidimetry; Western blot; and mRNA analyses.[10,29,39,90,108] Unfortunately, due to wide species variation in APP, few cross-reactive reagents are available. Assays for human APP have variable cross-reactivity.[31,38] Ideally, species-specific assays should be used. Although some commercially available ELISA kits are available to measure specific APP including CRP, SAA, and haptoglobin, most of these assays lack automation and are expensive. The ELISA methodology is best suited for batch analysis of many samples to make best use of technical labor and reagents. Some automated assays marketed for human APP have been validated for animal use, and few have been implemented for species-specific veterinary use.[29,60,61,122] Serum amyloid A appears to be conserved within mammalian species, and haptoglobin can be quantitated based on its universal reactivity with hemoglobin.[52,61,122] Standardization of APP assays has been a focus of international veterinary meetings and publications.[32,111] Species-specific controls should be considered when possible to allow for standardization of such assays between laboratories.[32,111] Other technical issues with APP testing include the variations in APP concentrations observed with different anticoagulants and interfering sample conditions such as hemolysis and lipemia.[10] Although APP are reported to be stable at –20 °C, for prolonged storage, –70 °C is recommended.[10]

Few studies in animals directly compare protein electrophoresis and APP ELISA assays in their ability to gauge the APR. In a study following rats for 21 d after dosing with complete Freund adjuvant, haptoglobin increased within 12 h.[43] This increase, nearly 700% of normal values, occurred concurrently with a significant decrease in albumin and increases in α2 and β globulins as quantitated by protein electrophoresis. Alterations in globulin, albumin, and haptoglobin concentration continued to be present until day 21, when normal levels were observed in all parameters. Therefore, although they are different methodologies, protein electrophoresis and APP ELISA could provide views of APR that reflect the same time course. The relative sensitivity of these methods may be better gauged by using less aggressive inflammatory stimuli or a more diverse inflammatory trigger, such as a chronically progressive disease condition.

## Applications of Acute Phase Proteins in Diagnosis and Prognosis

Before the advent of specific APP assays, monitoring the albumin:globulin ratio had been a standard in human and veterinary medicine to monitor inflammatory processes. Technology has expanded from protein electrophoresis to species-specific assays, which offer a more detailed examination of APP and their changes with disease progression. Triggering events of inflammation, infection, trauma, neoplasia, and stress alter APP levels in animal species; an extensive review of these publications is presented in Figure 3, which highlights APP changes in both experimental conditions and naturally occurring disease. For most species, traditional inflammatory agents such as croton oil and turpentine have been used to assess the types of APP produced and their magnitude and kinetics of expression. Changes in APP in experimental models of infection and naturally occurring inflammatory and infectious disease also have been assessed. Furthermore, several studies have demonstrated the utility of APP quantitation in monitoring neoplasia and stress.

| Triggering event | Animal | Significant changes in acute phase proteins | References |
|---|---|---|---|
| Infection | | | |
| | Cat | Serum amyloid A increased with feline infectious peritonitis infection | 44, 118 |
| | | α1-acid glycoprotein and haptoglobin increased with feline infectious peritonitis or feline immunodeficiency virus infection | 28 |
| | Chicken | Transferrin increased with *Staphylococcus aureus* infection | 12 |
| | | α1-acid glycoprotein increased with experimental infection with infectious bursal disease virus | 58 |
| | Cow | Haptoglobin, serum amyloid A, and fibrinogen increased with infections including bovine coronavirus and bovine adenovirus | 3, 40 |
| | Dog | C-reactive protein and serum amyloid A levels correlate with the severity of pyometra | 21, 85 |
| | | C-reactive protein and ceruloplasmin increased with babesiosis | 127 |
| | | C-reactive protein increased in dogs experimental infected with *Ehrlichia canis* | 110 |
| | | C-reactive protein increased in dogs naturally infected with *Leishmania* spp.; higher in symptomatic dogs | 79 |
| | Horse | Serum amyloid A increased with colic | 130 |
| | | Serum amyloid A increased with bacterial infection | 51, 55 |
| | Mouse | Serum amyloid P and haptoglobin increased experimental infection with *Trypanosoma* | 33 |
| | | Serum amyloid P increased in experimental malaria infection | 6 |
| | | Serum amyloid A and serum amyloid P increased with bacterial pneumonia | 99 |
| | Nonhuman Primate | C-reactive protein increased in macaques inoculated with *Bordatella bronchispetica* | 66 |
| | Ovine | C-reactive protein, haptoglobin, ceruloplasmin, and fibrinogen increased with bacterial infection | 112, 128 |
| | | Ceruloplasmin, fibrinogen, and haptoglobin increased and albumin decreased with yeast infection | 95 |
| | | Haptoglobin, serum amyloid A, and α1-acid glycoprotein increased in an experimental model of bacterial lymphadenitis | 34 |
| | Pig | C-reactive protein and serum amyloid A increased with experimental infection with the respiratory pathogen *Actinobacillus pleuropneumoniae* | 113 |
| | | Major acute phase protein and haptoglobin increased with pig coronavirus infection and wasting syndrome | 46 |
| | | C-reactive protein, serum amyloid A, and haptoglobin are valuable markers in natural infection with different porcine viruses and *Mycoplasma* infection | 91 |
| Inflammation | | | |
| | Cat | Serum amyloid A increased in acute pancreatitis in cats | 118, 119 |
| | | Serum amyloid A, α1-acid glycoprotein, and haptoglobin increased after surgery or injection with either LPS or turpentine | 71 |
| | Chicken | Transferrin increased with injection of croton oil or turpentine | 12, 124, 132 |
| | | α1-acid glycoprotein increased with injection of *Escherichia coli* LPS | 84, 117 |
| | Dog | C-reactive protein and α1-acid glycoprotein increased with injection of turpentine oil; 14-d time course for recovery | 48, 136 |
| | | Haptoglobin and fibrinogen significantly increased with hyperadrenocorticism | 8 |
| | | C-reactive protein correlated to severity and prognosis of acute pancreatitis | 78, 85 |
| | | C-reactive protein levels correlated with inflammatory bowel disease activity | 65 |
| | | C-reactive protein, fibrinogen, haptoglobin, and serum amyloid A increased with experimentally induced gastric mucosal injury | 7 |
| | | C-reactive protein increased after surgery; levels decrease by time of suture removal | 133 |
| | | C-reactive protein increased with chronic valvular disease | 103 |
| | | C-reactive protein increased with canine idiopathic polyarthritis; good prognostic indicator | 85, 88 |
| | | C-reactive protein increased with panniculitis | 85 |
| | Horse | Serum amyloid A increased and correlative to severity of joint disease | 63 |
| | | Serum amyloid A increased after surgery | 97 |
| | | Serum amyloid A increased after experimentally induced noninfectious arthritis | 56, 62 |

**Figure 3.** Summary of acute phase responses by triggering event.

The dichotomy of both rapid- and slow-responding APP provides an added dimension in clinical interpretation that is not readily seen with changes in other blood analytes and complete blood counts. In a large study, clinically ill cattle were assessed on the basis of signs, diagnostic testing, and postmortem findings.[53] The animals were categorized as experiencing either acute or chronic inflammatory processes. Serum amyloid A levels had 100% sensitivity and haptoglobin had 76% specificity in distin-

| Triggering event | Animal | Significant changes in acute phase proteins | References |
|---|---|---|---|
| | Mouse | Serum amyloid A increased with injection of ethanol | 98 |
| | | Serum amyloid A increased with injection of croton oil or *Aspergillus* antigen | 74 |
| | | Serum amyloid A, serum amyloid P, and α1-acid glycoprotein increased with injection of hepatotoxic agent or LPS | 45, 75, 131 |
| | | Serum amyloid A and serum amyloid P increased in IL2$^{-/-}$ mice which develop severe colonic disease | 22 |
| | Nonhuman Primate | Serum amyloid A increased in macaques with subclinical secondary hepatic amyloidosis | 54, 77 |
| | | Fibrinogen and α2-macroglobulin increased in rhesus monkeys treated with IL6 | 83 |
| | | C-reactive protein increased in different monkey species injected with turpentine oil | 67 |
| | Pig | C-reactive protein and haptoglobin increased with injection of turpentine | 35 |
| | | C-reactive protein, serum amyloid A, and haptoglobin increased in pigs with tail and ear bites and arthritis | 91 |
| | Rabbit | Serum amyloid A increased in an experimental model of non infectious chronic inflammation | 101 |
| | | C-reactive protein increased with injection of turpentine | 42 |
| | Rat | α1-acid glycoprotein, α2-macroglobulin, fibrinogen, and haptoglobin increased and albumin decreased with injection of turpentine | 9, 69, 108 |
| | | α2-macroglobulin increased 25-fold in rats subjected to surgery | 68 |
| | | α1-acid glycoprotein increased with injection of prostaglandin E2 for induction of fever | 129 |
| | | Haptoglobin increased with injection of complete Freund adjuvant or carbon tetrachloride | 43 |
| | | Haptoglobin increased and albumin decreased with injection of phosphodiesterase inhibitor | 27 |
| | Sheep | Ceruloplasmin and fibrinogen increased in animals with artificial pulmonary obstruction | 94 |
| Neoplasia | | | |
| | Cat | Serum amyloid A increased with lymphoma and malignant mesothelioma | 118 |
| | | α1-acid glycoprotein increased with lymphoma | 18 |
| | | α1-acid glycoprotein increased with carcinoma, sarcoma, and round cell tumors | 109 |
| | Dog | C-reactive protein useful in determining remission status in cases of multicentric lymphoma | 87 |
| | | C-reactive protein and haptoglobin increased with lymphatic neoplasia | 80 |
| | | C-reactive protein increased with hemangiosarcoma | 85 |
| | | C-reactive protein increased with mammary tumors; albumin decreased in metastasis | 120 |
| | | C-reactive protein, haptoglobin, and ceruloplasmin increased with hematologic and neoplastic diseases | 121 |
| | Mouse | C-reactive protein and haptoglobin increased in transgenic mice with intestinal adenoma | 37 |
| | | Serum amyloid A and C-reactive protein increased in model of fibrosarcoma | 14 |
| Stress | | | |
| | Cow | serum amyloid A increased in cows living in semiferal conditions with nutritional stress | 104 |
| | | serum amyloid A increased after exposure to complex stress (transportation, tie stall housing, slippery floors, social isolation) | 76 |
| | | serum amyloid A increased in calves after 3-h truck transportation | 5 |
| | | fibrinogen increased in calves with abrupt weaning | 49 |
| | Pig | C-reactive protein and haptoglobin increased after 48-h truck transportation; major acute phase protein increased after 24 h in both poor and superior conditions of truck transportation | 96, 106 |
| | | major acute phase protein increased after tail biting | 106 |
| | | C-reactive protein, haptoglobin, and major acute phase protein increased after repeated-bleeding protocols (sensitization to procedure) | 106 |
| | Rat | α1-acid glycoprotein and haptoglobin increased after exposure to tail shock | 23 |

**Figure 3.** Continued

guishing the groups. In comparison, neutrophil counts had much lower sensitivity and specificity (30% to 70%). In sheep, increases in haptoglobin had 85% sensitivity and specificity compared with 52% sensitivity and 75% specificity of WBC counts in those animals with naturally occurring bacterial infection.[112] In a retrospective evaluation of inflammatory conditions in dogs, APP showed significant changes in the absence of changes in the total or differential WBC count.[114] In response to steroid treatment, dogs with idiopathic polyarthritis showed a positive response to treatment (decreased symptoms) as well as significant decreases in CRP whereas total WBC count remained unchanged.[88] In a review of more than 900 cases of inflammation in dogs with various diseases, CRP concentrations were significantly correlated with disease, whereas only slight or no correlation was found with total WBC and band neutrophil counts.[85] In a cat with pancreatitis, SAA was increased with onset of symptoms whereas the WBC count was

normal.[119] With rapid resolution of the symptoms in this cat, SAA returned to normal levels, whereas the WBC count was just beginning to increase. In total, these findings are consistent with the expected delay in marked WBC changes due to the need to generate cells in the bone marrow, whereas APP concentrations can increase within hours.

Several studies have shown an association between APP levels and the severity of disease. In a study of naturally occurring babesiosis, CRP levels were significantly higher in dogs with severe or complicated disease.[127] In canine leishmaniasis, CRP was increased in symptomatic but not asymptomatic animals.[79] One group of investigators proposed a canine inflammatory bowel disease activity index, which was based on a panel of clinical symptoms relative to disease activity in canine inflammatory bowel disease.[65] Increased levels of CRP and α1-acid glycoprotein were associated with increasing activity indices of disease.

In these aforementioned studies as well as many others, APP have been demonstrated to change with a positive prognosis. Of note, the halflife of most APP is only 24 to 48 h. Thus, changes in APP may be more sensitive indicators of healing and resolution than other clinical tests. After surgery in dogs, CRP levels were found to decline and mirror the disappearance of clinical signs when the total WBC count continued to be elevated.[21,85] As mentioned earlier, CRP levels were decreased with a positive response to steroid treatment in dogs with polyarthritis.[88] In addition, after chemotherapy for multicentric lymphoma in dogs, CRP was within normal reference intervals in cases with complete remission.[87] In the study of canine inflammatory bowel disease, levels of CRP decreased with a positive response to therapy.[65] Haptoglobin, CRP, and SAA were monitored in dogs with pyometra before and after surgical sterilization.[21] Compared with those of normal dogs before surgery, CRP and SAA values in dogs with pyometra peaked within 24 h after surgery and then declined slowly in dogs that recovered without complications.

Several authors have suggested that a single APP should not be used exclusively to monitor a disease process. Instead, an APP index has been used in both human and veterinary medicine.[47] This index includes both positive and negative APP, as well as APP that increase both rapidly and slowly, thereby forming a comprehensive index that would correlate with the severity of the inflammatory process. A recent editorial on APP interpretation in companion animals summarized several integral points, including the need for an APP profile (for example, multiple positive APP).[11,126]

## Potential Novel Applications of Acute Phase Proteins in Laboratory Animal Medicine

Defining humane endpoints to experimental protocols remains a difficult task. Refinement of animal protocols is a primary goal of both investigators and institutional animal care and use committees, not only to gain valuable experimental information from animals prior to morbidity and mortality but also to meet the important obligation of minimizing pain and stress. Definition of humane endpoints often is driven by subjective measures of clinical condition, frequently in combination with weight loss. In many animal protocols, a moribund condition is used as a point of experiment termination. In some cases, a scoring of clinical and behavioral signs can be used to indicate that euthanasia is warranted. Such signs can include ruffling of the hair or coat, hunched posture, lethargy, decreased consumption of food or water, weight loss, diarrhea, bleeding, respiratory difficulty, impaired mobility, and unconsciousness.[86,125] In many cases, such clinical signs do not appear in all of the experimental animals, and some of these clinical signs may be transient and precede eventual recovery of the animal.[86] Another prevailing problem is that many of the variables monitored are subjective, and the accuracy of these assessments can vary among laboratory personnel and veterinary staff members. Moreover, investigators working with new models or newly created transgenic mice may not be able to rely on scoring systems determined previously on other models.[25,26] With the increasing use of animals in research, including the advent of transgenic mice and the increased need for models for translational studies, guidelines that are more objective are essential. APP have been proposed to be biochemical markers of stress, infection, and pain in laboratory animals.[89] Although no APP study has assessed this particular application specifically, a role for quantitating APP can be envisioned, given the broad increases that follow with triggering events including infection, inflammation, trauma, stress, and neoplasia, many of which are common conditions in experimental protocols involving the use of laboratory animals.

APP have been used to gauge stress with regard to animal well being. One investigator proposed a link among catecholamines, glucocorticoids, and production of APP in animals.[81] APP can indicate stress in pigs and calves. Pigs shipped under typical conditions had higher major acute phase protein (porcine), haptoglobin, SAA, and CRP than did pigs that were shipped under conditions that included the provision of sawdust, water, and feed.[96] Similar studies also demonstrated changes in APP in pigs, newly weaned calves, and cattle exposed to with new housing situations,[5,49,76,106] and in pigs that were unaccustomed to various handling procedures.[106] These results indicate the possible use of APR to monitor stress in laboratory animals and thus provide another marker of animal well being. Stressors that could be assessed in future studies of laboratory animals include physical environment (lighting, temperature, and noise), housing type, husbandry and handling, and shipping.

Another potential use of APP in laboratory animals is assessment of the general health of a colony. As indicated in Figure 3, APP are sensitive markers of inflammation and infection in animal species. In large animals, APP have been proposed as markers of herd health, through which a large group of animals may be assessed for food safety and herd management.[40,126] In 1 study, 2 groups of calves were examined over an extended time period. The group that had a higher incidence of disease also demonstrated significantly higher levels of APP.[40] Dogs maintained as laboratory animals had significantly lower CRP levels than did those kept as companion animals.[134,135] The authors hypothesized that the husbandry conditions and isolation of the laboratory animals minimized contact with potential inflammatory triggers.[10]

The use of APP to monitor rodent colony health may have limited value. Monitoring of APP levels would not be expected to be a primary tool used preferentially over traditional serologic and PCR screening methods; rather, APP assessment potentially would be an ancillary test. Additional studies should be performed before the implementation of APP monitoring in rodent colonies. For example, APP may be more suited to monitoring the health of individual animals. We have previously reported increased levels of α and β globulins in transgenic mice with der-

matitis.[137] In addition, APP might be used to screen animals (cat, dog, nonhuman primate, pig, and rabbit) during quarantine or before entry into experimental protocols. The nonspecificity of the APR has been proposed to offer a possible 'snapshot' of animal health and thus may reflect the presence of subclinical disease or other management issues.[30,105] APP may help to further characterize similar issues in laboratory animal colonies.

## Conclusions

The advantages and uses of APP assays are well supported in the human and veterinary medical literature. Unfortunately, because of the practical limitations of current technology, clinical application of APP analysis is not widespread. Continuing challenges include the need for automated assays and standardization of tests across laboratories. However, many potential uses are possible for APP and APR in laboratory animal medicine. Exceptionally sensitive yet advantageously nonspecific markers of diverse inflammatory etiologies, APP are excellent candidates through which to better study animal models of disease, monitor animal health, and objectively assess animal wellbeing.

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