Haptoglobin and plasma viscosity as markers of acute phase reactions in red deer *Cervus elaphus.*

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SUMMARY.

A protein band found intermittently in red deer during hybrid or parentage testing is identified as haptoglobin. Evidence is presented that its presence is related to an acute phase reaction in the animal concerned, as indicated by plasma viscosity and/or fibrinogen. Reporting of elevated haptoglobin detected during routine blood typing may therefore alert the practitioner to the presence of occult tissue damage, inflammation or infection in potentially valuable animals.

INTRODUCTION

The technique of gel electrophoresis used to identify inherited protein variation in deer for parentage testing and hybrid identification, also detects non-inherited variation in an unidentified protein located in the gamma globulin region of plasma (Tate and Dratch, 1987) In this paper we report the identification of the protein as haptoglobin and examine the relationship between its occurrence and markers of acute phase reactions in deer

Within hours of the occurrence of tissue damage, microbial infection or inflammation a number of plasma proteins increase in concentration in the systemic circulation (Dinarello 1984) These acute phase reactants include fibrinogen, haptoglobin, ceruloplasmin, C-reactive protein, serum amyloid A and alpha-1 anti-trypsin The changes appear to be mediated by tumour necrosis factor via interleukin-1 and probably other intermediaries (Tracey et al, 1988) In man C-reactive protein and serum amyloid A show marked increases compared to the others, whereas in ruminants haptoglobin shows the more marked increase (Conner et al 1986) In a recent study on sheep comparing serum ceruloplasmin, fibrinogen, serum haptoglobin and leukocyte concentrations during induced acute phase reactions, haptoglobin was found to show a very marked rise comparatively, in some cases greater than 60-fold (Pfeffer, and Rogers, personal communication)

METHODS

The alkaline polyacrylamide electrophoresis method used to resolve haptoglobin is that described by Gahne et al , (1977) with the modifications of Tate and Dratch (1987)

The methods for fibrinogen and plasma viscosity have been presented previously (Cross, 1987) Reference values for plasma viscosity and fibrinogen on mixed age adult hinds were derived from 70 healthy animals with no clinical history of recent illness, negative lymphocyte transformation for both avian and bovine tuberculosis and eosinophils below 0 5 x 10^9/L 95% reference limits of 1 39-1 61 centipoise (cp) for plasma viscosity measured at 25^{0}C., and 170 to 340 mg/dL for fibrinogen were obtained When comparing means, data sets were checked for normality using Lilliefors modification of the Kolmogorov-Smirnov test (Lilliefors, 1967) Fishers exact test was used on the data in table 1

SOURCES OF DEER

Most of the serum samples were obtained from 10 ovariectomised mixed age adult hinds during a longitudinal study in which they were bled at intervals during a 4 week period On the basis of plasma viscosity measurements, one of these animals appeared to be undergoing a severe occult acute phase reaction which responded to antibiotic, and another 2 had slightly elevated plasma viscosities at different times during the 4 week period The remaining specimens were collected from mixed-age hinds from a herd with a high level of bovine tuberculosis, specimens being collected before and after skin testing It has been shown that skin testing initiates at least some elements of an acute phase response in a proportion of such animals (Cross, 1987) It was therefore anticipated that they would provide an excellent acute phase response model without the need for artificial induction of inflammation

RESULTS

Three pieces of evidence identified the protein band as haptoglobin Firstly, the band was a high molecular weight glycoprotein, secondly, the addition of haemoglobin to plasma samples prior to electrophoresis reduced the electrophoretic mobility of the band and induced peroxidase activity, thirdly, the protein band was bound strongly by rabbit antihuman haptoglobin sera

The mean plasma viscosity in haptoglobin positive animals was 1 78 cp, and in haptopglobin negative animals was 1.55 cp (p <0 0001) These results are summarised in table 1

	Haptoglobin raised	Haptoglobin negative	p
Plasma viscosity:			
raised	20	4	<0.001
normal	2	55	

Table 1 Haptoglobin is raised in deer with an acute phase reaction as indicated by plasma viscosity

The acute phase reactant fibrinogen was measured in 10 specimens, 7 with raised haptoglobin having a mean fibrinogen of 620 mg/dL, while 3 showing no haptoglobin had a mean fibrinogen of 269 mg/dL ($p = 0.023$)

Specimens were taken just prior to skin testing and 72 hours later from 5 adult hinds known to be tuberculous from lymphocyte transformation results, and having tuberculous lesions at post-mortem 3 of the 5 showed an increase in fibrinogen and plasma viscosity in response to skin testing, and all 3 showed a clear increase in the haptoglobin band at 72 hours compared to the pre-skin test specimen The 2 animals showing no fibrinogen or plasma viscosity increase at 72 hours did not show any haptoglobin increase

Results in an animal with a high plasma viscosity responding to antibiotic are shown in fig 1 An infection was suspected in this hind owing to the high plasma viscosity with no apparent cause In response to an antibiotic injection, the plasma viscosity dropped steadily to normal, indicating that the infection was resolving with concurrent disappearance from the circulation of acute phase reactants In parallel, semi-quantitative estimates of the haptoglobin concentration were made by one of us without knowledge of the viscosity results, and followed a similar path

DISCUSSION

Plasma viscosity measurement offers a precise and sensitive method for screening several acute phase reactants (Hawkey, 1971) and has been used to monitor inflammation in red deer (Cross, 1987)

Plasma fibrinogen is well-known as a marker of acute phase reactions and has recently been the subject of a retrospective survey in mammals including deer, which illustrated that it is an effective test for the presence of bacterial infection in Artiodactyla (Hawkey and Hart, 1987)

It is clear from the results given in this paper that the increase in haptoglobin as detected by the density of the band on electrophoresis is commonly found in deer with signs of acute phase response (raised fibrinogen and/or plasma viscosity) and rarely present in animals showing no such signs In the case of the animal treated with antibiotic, an initially high haptoglobin level fell away to zero as the infection resolved Skin tested animals with no visible haptoglobin band prior to skin testing showed high levels post skin testing along with other signs of acute phase response

Thus haptoglobin is a useful indicator of acute phase reactions in red deer, and has the advantage of being incidentally derived from phenotype investigations on potentially elite herds When interpreting such results, it is necessary to be aware of the possible effects of skin testing for tuberculosis

REFERENCES

Conner J G, Eckersall P D, Doherty M, and Douglas T A (1986) Acute phase response and mastitis in the cow Res in Vet Sci 41 126-128

Cross J P, (1987) Haematology-based prediction of lesion status in bovine tuberculosis of farmed red deer Proc Deer Course for Veterinarians, 4 147-154

Dinarello C A, (1984) Interleukin-1 and the pathogenesis of the acute phase response New Eng J Med 311 1413-1418

Gahne G, Juneja R K, and Gromulus J, (1977). Simultaneous phenotyping of transferrin, post transferrin, albumin and post albumin in the blood plasma of cattle Animal Blood Groups and Biochemical Genetics 8 127-137

Harkness J (1971) The viscosity of human blood plasma its measurement in health and disease Biorheology 8 171-193

Hawkey C M, and Hart M G, (1987) Fibrinogen levels in mammals suffering from bacterial infections Vet Record 121 519-521

Lilliefors H W, (1967) On the Kolmogorov-Smirnov test for normality with mean and variance unknown J Am Statist Assoc 62 399-402

Tate M L, and Dratch P A, (1987) Inherited protein variation and parentage testing in farmed red deer Proc N Z Society of Animal Production(In press)

Tracey K J, Lowry S F, Cerami A (1988) Cachectin A hormone that triggers acute shock and chronic cachexia J Infect Dis 157 413-420

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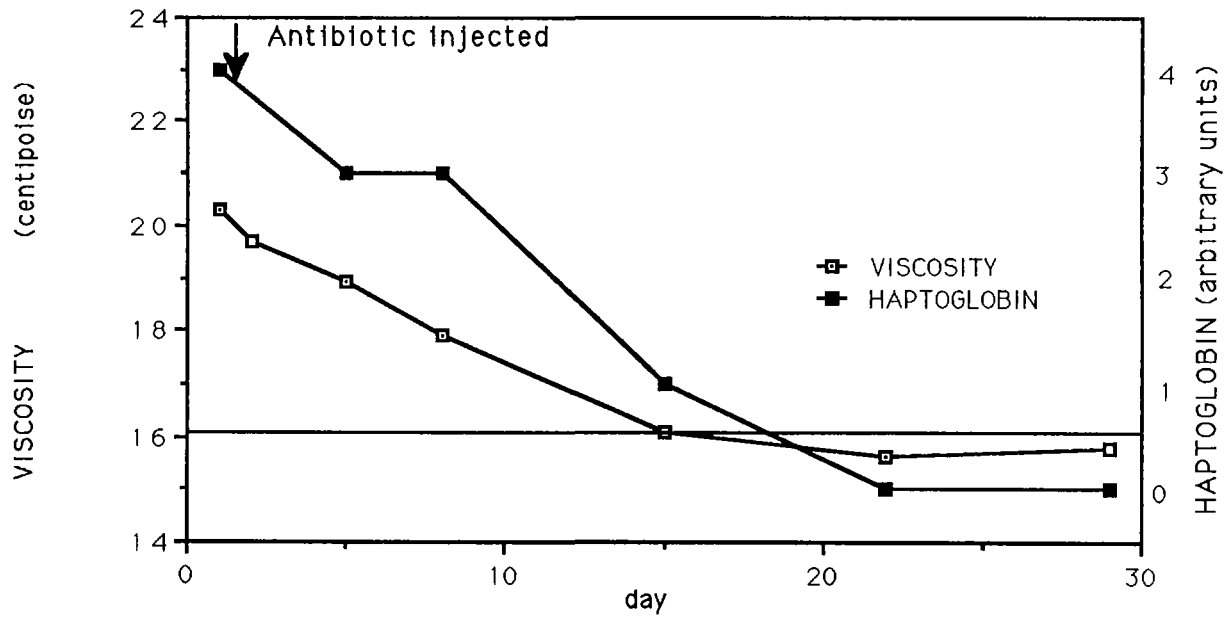

Fig.2 Plasma viscosity and haptoglobin during a resolving infection in a red hind.