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ISOLATION AND CHARACTERIZATION OF EQUINE HAPTOGLOBIN, AND ITS EVALUATION AS AN ACUTE-PHASE REACTIVE PROTEIN IN HORSES

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Haptoglobin (Hp) was isolated from equine serum by ammonium sulphate precipitation, anion-exchange chromatography and gel filtration. Purified Hp was characterized by forming a complex with equine hemoglobin and its immunological cross-reaction with anti-human Hp.

Equine Hp migrated to the alpha-2 globulin region under cellulose acetate electrophoresis. Equine Hp contained two fractions with molecular weights of 108,000 and 105,000, and each fraction was composed of two subunits. The fraction with a MW 108,000 had one type of subunit which had a molecular weight (MW) of 50,000, and the other fraction with a MW of 105,000 had two types of subunits which had MWs of 47,500 and 50,000. These subunits of equine Hp were glycosylated. The isoelectric point of equine Hp was estimated to be about 4.1-4.3. Equine Hp had about 950 amino acid residues and its composition was similar to that of human Hp. Spur formation in double immunodiffusion test confirmed that equine Hp shared a common antigenic region with human Hp.

The single radial immunodiffusion method was used for quantitative measurement of Hp in equine serum. In clinically normal horses, the concentration of serum Hp was highest in newborn foals (7.05 ± 2.07 mg/ml) and was maintained at a high level (5.12 ± 2.34 mg/ml) until 12-month-old. Its concentration then showed a tendency to decrease with ageing. Normal Hp values are 5.25mg/ml in foals (≤ 12 -month-old), 2.19mg/ml in adult horses (≥ 18 -month-old) and 3.62mg/ml in all horses. Serum Hp concentration in mares during the peri-natal period, in comparison with the normal value for adult females, was high for 4 months pre-partum and showed a passing increase at delivery. It decreased at two weeks post-partum and returned to the normal value within one month of delivery. In horses with experimentally induced inflammation, serum Hp concentration began to increase immediately after the treatment and reached a maximum, 1.5 to 9 times higher than pre-treatment levels, on the 4th to the 6th day. It then returned to the pre-treatment value after 2 to 4 weeks. In horses with clinical signs of inflammatory disease, the serum levels of Hp in most cases were statistically high compared with the normal value.

Hp was therefore concluded to be an acute-phase reactive protein in horses.