Plasma Protein Response in Experimental Inflammation in the Dog*

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Summary. The changes in plasma protein concentration in experimental inflammation in dogs were studied. Arthritis, turpentine injury, thrombophlebitis, proctitis and cholecystectomy were produced in the animals. The orosomucoid concentration rose to values higher than those seen in man. C-reactive protein, fibrinogen, haptoglobin, ceruloplasmin and β1C-globulin increased in concentration in the same way as in man. In contrast with what is seen in human beings, α1-antitrypsin did not increase and α2-macroglobulin was clearly reduced during the inflammation.

Key words: Inflammation — Plasma proteins — Alpha globulin — Acute phase reaction — Immunoassay.

Certain plasma proteins increase in concentration in the presence of inflammation in man and other mammals (Crockson et al., 1966; Williams and Wemyss, 1961). It has been shown that in several proteins this is due to an increase in the rate of their production (Maung et al., 1968; Koj, 1968). In many investigations the plasma protein concentration has been determined indirectly from measurement of the amount of protein-bound hexose and hexosamine (Ashton et al., 1970). Most investigations with specific determination of several proteins have been carried out on man.

Little or nothing is known of the biological function of the increase in most of the plasma proteins in inflammation. By comparing the patterns of plasma protein changes in the presence of inflammation in different species it would be possible to find out which protein or proteins are regularly involved in the inflammatory response and which are therefore probably of greatest biological significance.

The purpose of the present investigation was to study the changes in the concentration of a series of proteins in inflammation in the dog.

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The inflammatory responses of the 9 proteins selected are well known in man. C-reactive protein (CRP), haptoglobin, fibrinogen, orosomucoid, α₁-antitrypsin, ceruloplasmin and β₁C-globulin are known as "acute phase reactants". Albumin and α₂-macroglobulin (α₂-M) were selected to have a low molecular weight and a high molecular weight protein for comparison. Experimental inflammation was used to permit investigation of the inflammatory response of the plasma proteins per se. The purpose was to identify any general pattern in the dog differing from that in man.

Methods

Preparation of Antigen and Antisera

Dog orosomucoid was purified according to the method used previously for human orosomucoid (Lange, 1967). At electrophoresis in polyacrylamide gel, pH 8.3, the protein migrated as a homogenous band. The glucosamine content was determined with JEOL Amino Acid Analyzer JLC-5 AH to 17% (6-hour hydrolysis in 4 M HCl, 100°C).

Dog haptoglobin was prepared by precipitation with ammonium sulphate and the fraction collected between 1.9 and 2.2 M was dissolved and purified further by chromatography on DEAE-Sephadex. It was finally purified by preparative electrophoresis (Johansson, 1972). The purified protein, which migrated as a homogenous component in the α₂-region on agarose gel electrophoresis, pH 8.6, formed a complex with purified dog haemoglobin and then migrated slower on electrophoresis.

Dog β₁C-globulin was prepared from freshly obtained dog serum in the way described previously for human serum (Müller-Eberhard et al., 1960). On agarose gel electrophoresis the protein migrated as a homogenous band and at different rates according to the presence or absence of Ca++. In the buffer.

Dog ceruloplasmin was purified in the way described for human serum previously (Deutsch, 1960), but with the following modifications. After DEAE-cellulose chromatography the ceruloplasmin was identified in the elution tubes by electroimmunoassay (Laurell, 1972) with rabbit anti-human ceruloplasmin, which cross-reacted with dog ceruloplasmin. After the first chromatography on DEAE-cellulose the fraction was precipitated with ammonium sulphate to 2.3 M. The second chromatography was not followed by ammonium sulphate precipitation. The first four elution tubes contained ceruloplasmin without any contaminant demonstrable on agarose gel electrophoresis. The purified protein was used for immunisation and the antisera obtained revealed antibodies against two proteins when checked by crossed immunoelectrophoresis (Ganrot, 1972a). One of the precipitates was identified as ceruloplasmin by demonstrating oxidase activity on treatment with p-phenylenediamine (Holmberg and Laurell, 1951).

Antiserum were obtained by immunising rabbits. 0.1—1 mg of each of the above purified proteins was emulsified with an equal volume of Freund's complete adjuvant and injected subcutaneously into rabbits. 2—3 weeks later the animals were given a booster dose of the same size as the initial dose and 2 weeks later blood was obtained from the rabbits.

Rabbit anti-dog CRP (Kindmark, 1972), rabbit anti-dog α₂-M (Ganrot, 1968), rabbit anti-dog α₁-antitrypsin and rabbit anti-dog fibrinogen (Ohlsson, 1971) were available at the laboratory.