IMMUNOLOGY

Relationship of the Anti-complementary Effect of Fowl Serum to its own Haemolytic Activity

THE marked anti-complementary effect of fresh fowl serum on guinea-pig complement and its reduction by heating to 56° C are well known[1,2]. Treatments of fowl serum by methods other than heating which reduced this effect also resulted in a decrease in its own haemolytic activity[3], suggesting that the anti-complementary effect of fowl serum may be related to its own complement content. More recently, it has been shown that the euglobulin fraction of fowl serum, corresponding to the mid-piece or first component of fowl complement, is the fraction which reduces the haemolytic titre of guinea-pig complement[4,5]. This heat-labile euglobulin fraction appears to be the same as the 'normal chicken factor' reported to be necessary for the specific fixation of guinea-pig end-piece by fowl antibody/antigen complexes[4,6]. Its anti-complementary effect on guinea-pig complement was also mentioned, but it was stated that this could be removed by dissolving the fraction in heated normal fowl serum to give a 'concentrated normal chicken factor'[5]. Orlans, Rose and Clapp[3] investigated this factor and found that, with certain concentrations of fowl serum, it could produce reductions in the haemolytic activity of guinea-pig complement, simulating specific fixation by antigen/antibody mixtures. We have now compared the effects of fowl serum, before and after various treatments, on the haemolytic activities of both fowl and guinea-pig complements.

The results are shown in Table 1. The effects of the treated fowl sera (FS) on both guinea-pig (g-pig) and fowl haemolytic activities were strikingly similar, suggesting that they were due to a component or components of fowl complement. Most convincing was the marked reduction of the anti-complementary effect after the absorption of the fowl serum with a specific fowl antibody/antigen precipitate in which the antigen was bovine serum albumin (known to have no effect on complement). Table 1 also shows that the anti-complementary factor of normal fowl serum is concentrated in the euglobulin fraction and is heat-labile; and also that this fraction has a similar inhibitory effect on lysis by fowl complement. Fowl pseudoglobulin fractions increase the haemolytic activity of fowl complement and are only slightly inhibitory to guinea-pig complement. The effects of fowl eu- and pseudo-globulin fractions on the haemolytic activity of fowl complement are given in greater detail elsewhere[7].

Table 1. EFFECT OF VARIOUS TREATMENTS OF FOWL SERUM ON ITS HAEMOLYTIC AND ANTI-COMPLEMENTARY ACTIVITIES

Treatment of fowl serum		Anti-complementary effect on guinea-pig C[1]	Effect on haemolytic activity of fowl C[1]
None		Strong Lysis reduced by 76·5% in mixture of 1 part FS and 4 parts g-pig C[1]	
Heat 56° for 30 min		Weak Lysis reduced by 10% in mixture of 1 part FS and 4 parts g-pig C[1]	Reduced or enhanced depending on concentration[9]
Absorption with specific fowl antibody/antigen ppt. (BSA-anti-BSA)		Weak Lysis reduced by 13·5% in mixture of 1 part FS and 4 parts g-pig C[1]	Reduced by 50% (see reference 10, Table 4)
Fractionation by dialysis	Euglobulin	Very strong Lysis reduced by 98% in mixture of equal parts euglobulin solution and g-pig C[1]	Reduced by 46% in mixture of 1 part euglobulin and 1·5 parts FS
	Heated euglobulin (56° for 30 min)	Very weak Lysis reduced by 5% in mixture of equal parts euglobulin solution and g-pig C[1]	Slightly reduced by 24% in mixture of 1 part euglobulin and 1·5 parts FS
	Pseudoglobulin	Medium Lysis reduced by 27% in mixture of 1 part pseudoglobulin solution and 4 parts g-pig C[1]	Enhanced by 45% in mixture of equal parts of pseudoglobulin and FS

Methods are given in references 7 and 9.

A fowl serum macroglobulin which is present in the euglobulin fraction of fowl serum has already been described[8]. This macroglobulin appears to react with complement and resembles both conglutinin and rheumatoid factor in that it can be removed, at least in part, by a specific precipitate; but, as far as its contribution to specific precipitates is concerned, it is heat-stable[7].

The results suggest a method for removing the anti-complementary effect of fowl serum on guinea-pig complement by dialysis against low ionic strength buffer to remove the euglobulin. The resulting pseudoglobulin preparation contains the greater part of the antibody when fractionation is done at approximately neutral *p*H, and fixes fowl complement to the same extent as the corresponding whole antiserum[7]. However, this pseudoglobulin antibody does not fix guinea-pig complement.

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Serum Proteins of Germ-free Rats fed Water-soluble Diets

THE germ-free animal with its more controlled environment and reduced reticuloendothelial system has become a valuable tool in microbiological and immunological investigations. In the germ-free rat a deficit in immune proteins causes low levels of serum β- and γ-globulins[1]. However, the animal is still subject to the variable reticuloendothelial system stimulating factors occurring in the steam-sterilized diets generally used. This communication presents data on serum proteins of germ-free rats fed a chemically defined, water-soluble 'antigen free' diet which was filter-sterilized. This type of diet, composed almost entirely of low-molecular-weight compounds and free of material of bacterial origin, should further reduce the level of reticuloendothelial system activity because of the absence of stimulation caused by high-molecular-weight substances and by killed and disintegrated microbial agents.

Germ-free Lobund Wistar rats, within one day after birth, were placed in a hand-rearing isolator and force-fed the water-soluble diet via special nipples at 0·5 h intervals[2]. At approximately 17 days of age self-feeding was started which continued until the animals were killed at an age of 2·5 months. All animals were maintained in wire-bottom cages without bedding.

Cellulose acetate electrophoretic analyses of sera from germ-free and conventional rats fed practical type diet *L*-462 (ref. 3) and from germ-free rats fed the water-soluble formula are shown in Table 1. Total serum protein of the rats fed the water-soluble diet was 5·68 g per cent, comparable with the value of 5·49 g per cent for the germ-free rats maintained on diet *L*-462. Comparison of the serum protein pattern of germ-free and conventional rats thus obtained generally shows no