levels of IgG bearing lymphocytes in aged donors were higher than in young controls at the corresponding experimental stages. Figure 1 reports the effect of blood serum, both from young and aged donors, on the percentage of IgG bearing cells in a group of homologous young normal lymphocytes previously submitted to the 'shedding' procedure. Only after incubation with 'aged' serum was such a value enhanced at a significant level. Figure 2 depicts the values of C1qbinding activity of blood sera from young and aged donors: 19 out of 30 'aged' sera exhibited a significant binding activity, whilst no positivity was recorded among the 'young' sera.

2 main results were observed concerning Ig bearing peripheral lymphocytes after in vitro culturing. An increase in the percentage of IgM bearing cells took place both in young and in aged donors. A similar increase is evident when lymphocytes are stimulated with PHA[13,14]; thus we assume that a better expression of the true receptor molecules on B-lymphocytes may be due to their stimulation in vitro. It is possible that, even if to a lesser degree, such a process is operating also during the short in vitro culturing performed (in presence of FCS) in our 'shedding' process. The second effect observed was a decrease in the percentages of IgG bearing lymphocytes both in young and in aged donors. Such a result suggests that serum born IgG are passively attached at least on a fraction of peripheral lymphocytes. The possibility that both shedding and regeneration processes of IgG receptors may exhibit age-related changes seems to be unlikely if we take into account the effect of the incubation with blood sera. Thus the age-associated differences in the percentage of IgG positive lymphocytes may be attributed to a higher concentration of such 'absorbable' IgG in blood serum from aged subjects. The effect of serum from aged donors on lymphocytes from young subjects is consistent with such a possibility. 2 possible explanations can be advanced; firstly, anti-lymphocyte autoantibodies may be present in blood sera[7,15–17]. However the blood sera employed were devoid of such antibodies, at least as far as they are detectable by the lymphocytotoxicity test. An alternative explanation assumes that antigen-antibody complexes may be bound on lymphocytes through their Fc or Complement receptors[18–20]. Should this be the case, the concentration of such complexes would be higher in the sera from aged donors. Though not conclusive, the results obtained in the ^{125}I-C1q binding test are consistent with our second hypothesis.

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A haemagglutinin in the tissue fluid of the Pacific oyster, Crassostrea gigas, with specificity for sialic acid residues in glycoproteins[1]

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Summary. An agglutinin for human red cells has a specificity for sialic acid and a high affinity for bovine salivary glycoprotein. Digestion of the glycoprotein with Pronase or neuraminidase indicated that binding of sialic acid to receptors in the agglutinin is the first step in the mechanism of formation of a stable complex between ligand and receptor.

Carbohydrate-binding proteins, the lectins, have been obtained from both plant and animal tissues[2]. The specificity has usually been determined by competitive-binding studies involving the ability of monosaccharides or their derivatives to inhibit the agglutination of red blood cells by the lectin. At the same time, it has been demonstrated that glycoproteins or glycopeptides have in many cases a significantly greater affinity for the lectin than the corresponding monosaccharide[3,4]. The reasons for this important difference remain to be defined. The present report is concerned with the occurrence and properties of the agglutinins present in the haemolymph and tissue fluids of the Pacific oyster with particular emphasis on a lectin that exhibits this difference with regard to free sialic acid and that bound as the terminal carbohydrate residues in some glycoproteins.

Tissue fluid, consisting mainly of haemolymph, was obtained by collecting the clear supernatant remaining after centrifugation (30,000 × g for 10 min) of the freshly dissected soft tissues of Crassostrea gigas. This fluid agglutinated all red blood cells of the species tested as well as other cell types including bacteria. In a standard plate assay (equal volumes of diluted tissue fluid and of a 2% v/v suspension of red blood cells in saline) typical titres obtained by serial dilution of the tissue fluid were 1/1024 and 1/256 using equine and human red blood cells respectively. These agglutinins are subsequently referred to as Gigalin H (human) and Gigalin E (equine). They are separate entities as judged by cross-absorption experiments and by the preferential absorption of Gigalin H from tissue fluid by an affinity column prepared by the reaction of CNBr – activated Sepharose 4B (Pharmacia) with a preparation of bovine salivary gland glycoprotein (BSG). The bound agglutinin could subsequently be displaced by the use of a gradient of increasing ionic strength in the eluant buffer. In addition, after treatment with neuraminidase, human red cells were not aggluti-

1 We wish to thank Dr P. Walne, Conwy Laboratory, MAFF, for generous supplies of Crassostrea gigas. One of us (SWH) wishes to thank the Natural Environment Research Council for financial support.
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nated by Gigalin H whereas this treatment of horse red cells had no detectable effect on the reaction with Gigalin E.

The possibility that sialic acid was implicated in the reaction of Gigalin H with human red cells was also suggested by a comparison of the effect of various substances as inhibitors of haemagglutination. A variety of neutral hexoses, pentoses, and uronic acids were without effect. BSG was the most effective inhibitor (figure 1, table). The amount of this glycoprotein, expressed in terms of sialic acid, was about 16,000 times less than that required for either free N-acetyl neuraminic acid or N-acyl neuraminyl 2 → 6 N-acetyl galactosaminitol to inhibit haemagglutination by 50% (table). Moreover the amount of bound sialic acid present in the red blood cells in the assay system (figure 1) was about 300 times that present in the amount of BSG used in the assay to inhibit haemagglutination by 50% (table). In contrast to these results it has been shown[4] that the agglutination of horse red blood cells by a partly purified preparation of Limulin, a lectin of the horse-shoe crab Limulus polyphemus, is inhibited to the same extent by both BSG and by the derivative of the carbohydrate prosthetic group, N-acyl neuraminyl 2 → 6 N-acetyl galactosaminitol – an observation leading to the conclusion[4] that the ligand specificity of Limulin is for the complete carbohydrate prosthetic group of the glycoprotein.

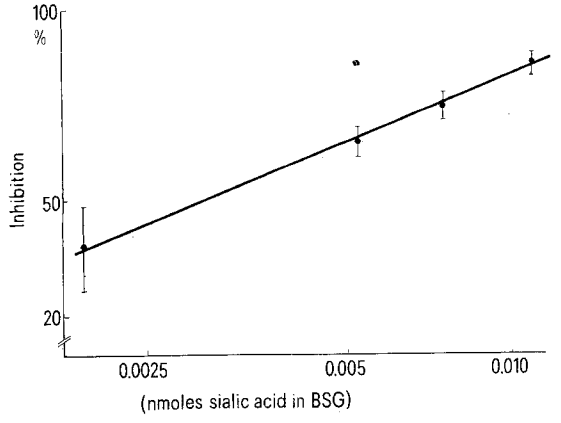

Fig. 1. Effect of BSG on the agglutination of red blood cells by Gigalin H. Tissue fluid was diluted with a marine saline[11], to a final titre of 1/32 in a standard haemagglutination plate assay. 0.1 ml of a solution containing BSG was mixed with 0.1 ml of the diluted tissue fluid. After 30 min, 0.1 ml of a 2% v/v suspension of group 0 human red blood cells was added (about 1 nmole of bound sialic acid by analysis[6]). The percentage of red blood cells agglutinated, ± SD, n = 4, in a 2 h period was determined by a modification of a spectrophotometric method[12].

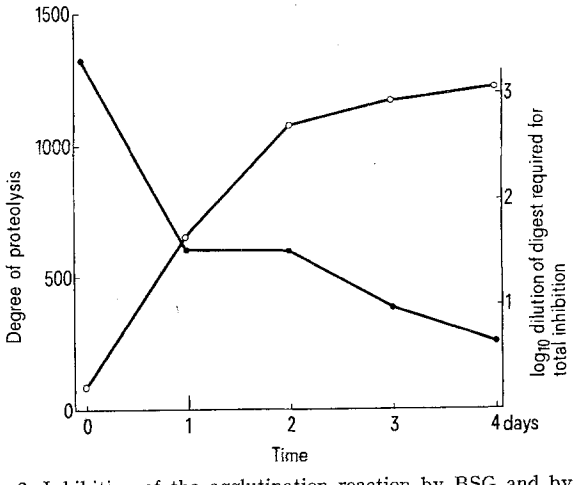

Fig. 2. Inhibition of the agglutination reaction by BSG and by a Pronase digest of BSG. 50 mg of BSG was digested with 2.5 mg of Pronase (Calbiochem) added in 4 equal portions at 24 h intervals[5]. Samples were removed at zero time, at 24 h intervals and the reaction was stopped by heating at 100 °C for 10 min. The effect of the digest on the agglutination reaction between group 0 human red blood cells and Gigalin H (●) was determined as the highest dilution of the digest that effected complete inhibition under the conditions given in the table; the end-point being judged visually. An approximate measure of the degree of proteolysis was made by determination of the increase in the capacity of the digest to yield a blue colour in the ninhydrin reaction[17]. The results are expressed as glycine equivalents corrected for both substrate and enzyme controls (○).

Digestion of BSG with an endopeptidase of broad specificity such as Pronase resulted in a very significant decrease in the affectiveness of the digest to act as an inhibitor of haemagglutination (figure 2). This was most marked after digestion for 24 h, corresponding to about a 95% decrease in activity. After exhaustive digestion for 96 h the residual glycopeptides were still more effective inhibitors of haemagglutination than free N-acetyl neuraminic acid. Under similar conditions, Carubelli, Bhavanandan and Gottschalk[5], showed that treatment of BSG with Pronase resulted in the formation of a mixture of glycopeptides with an average composition of 4 amino acid residues to 1.3 residues of N-acetyl galactosamine. Treatment of BSG with neuraminidase (10 mg BSG in 0.01 M acetate buffer, pH 5.5 containing 0.002 M Ca^{2+}, was digested with 1 IU enzyme at 37 °C for 60 min): the reaction was stopped by heating at 100 °C for 10 min reduced the inhibitory titre of the digest by about 90%. Analysis of the digest for free and bound sialic acid[6] showed that about 75% of the terminal sugar had been released from the glycoprotein.

It is generally accepted that BSG is microheterogeneous and consists of a polypeptide chain with several hundred relatively simple carbohydrate prosthetic groups[7], principally N-acetyl and N-glycolyl neuraminyl 2 → 6 N-acetyl galactosamine[8], linked to the protein core by O-glycosidic bonds involving the galactosamine and hydroxyamino acid residues. On this basis, it is possible to conclude from the present results that Gigalin H must have multiple binding sites with an affinity for N-acyl neuraminic acid residues. At the same time, the results of digestion of BSG with Pronase (figure 2) would indicate that the orientation in space and constraints on the movement of the terminal sialic acid residues in the prosthetic groups distributed along the intact polypeptide chain are of crucial importance for the stability of the BSG-Gigalin H complex. Similarly, the relative effectiveness of the sialoganglioside as an inhibitor of the haemagglutination reaction (table) may be attributed to the orientation in space of the sialic acid residues in the water phase at the lipid-water interphase of the micelle of the liposaccharide. The binding of sialic acid residues to receptor sites in Gigalin H may thus be an essential but only a first step in the overall mechanism of complex formation. When the ligand is a soluble or membrane-

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Effect of various compounds on the agglutination of human red blood cells by Gigalin H

Compound	nmole required to inhibit agglutination by 50%
N-acetyl glucosamine	1350
N-acetyl galactosamine	1120
N-acetyl neuraminic acid	57
N-glycolyl neuraminic acid	56
Sialolactose (N-acetyl neuraminyl 2 → 6, galactose 1 → 4, glucose)	44*
BSG-disaccharide (N-acyl neuraminyl 2 → 6, N-acetyl galactosaminitol)	47*
Disialoganglioside (sialosyl galactosyl – N-acetyl galactosaminyl (sialosyl) – lactosyl-ceramide)	1.7*
Fetuin	1.3*
BSG	0.0035*

* Expressed in terms of content of sialic acid. The BSG-disaccharide was obtained by treatment of BSG with alkaline borohydride[13] followed by ion-exchange chromatography[14]. Sialolactose was obtained from Sigma and the 2 → 6 isomer was isolated from the mixture of 2 → 3 and 2 → 6 isomers bypa per chromatography[15]. Disialoganglioside was obtained from PL Biochemicals and purified by thin layer chromatography[16]. All other compounds were obtained from Sigma and were used without further purification.

bound macromolecule, multiple binding of sialic acid residues to groups or clusters of receptor sites may, by analogy with other ligand-receptor models of the allosteric[9] or induced-fit[10] type, result in a conformational transition in Gigalin H with the subsequent development of other cohesive forces that stabilize the complex at equilibrium.

The data reported here are insufficient to justify further discussion. Direct studies on equilibrium binding kinetics involving the purified lectin will be necessary to characterize the specific and other cohesive forces that together determine the affinity constant of complex formation and to define the potential use of Gigalin H as a membrane probe.

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Activation of complement by trypanosomes[1]

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Summary. Factors exhibiting anti-complementary activity released from trypanosomes after incubation at 20 °C were described. The active material was shown to consume the first component of bovine complement. While the anti-complementary factor(s) from T. lewisi could activate bovine, human and guinea pig complement, the factor(s) from T. congolense was observed to activate bovine complement, but not guinea pig and only slightly human complement. The roles of complement activating factor(s) of trypanosomes in the pathology of the disease are discussed.

Decreased levels of hemolytic complement have been reported in trypanosome infections of man[2] and cattle[3]. This drop in hemolytic complement coincides with a decline in parasitemia, the appearance of complement fixing antibody to the organism and the onset of anemia[4]. In addition, alternate pathway fixation of complement to glomeruli in this disease has been reported by Nagle et al.[5]. Considering these parameters, it would appear that the complement levels correspond closely to immune complex formation and to production of antibody to antigens associated with erythrocytes. As there is evidence to suggest that the recorded decline in parasitemia is at least partly a result of adherence of the organism to blood vessel walls and invasion of solid tissues by the parasite[6], the blood parasite count can therefore not be solely responsible for the loss of complement activity by immune complex formation. Such arguments would indicate the existence of other mechanisms of complement consumption in this disease.

We present evidence to show that Trypanosoma congolense (strain TREU 112) can activate bovine complement directly by the classical pathway of consumption. In addition, T. lewisi was shown to activate not only the bovine complement cascade but also those of human and guinea pig sera.

Material and methods. T. congolense and T. lewisi were cultured in rats and purified from their blood by the method of Lanham and Godfrey[7]. Freshly purified T. congolense were suspended in 0.04 M phosphate buffer pH 8.0 containing 0.04 M NaCl and 1.0% glucose (PSG)

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