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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to a final concentration of 1×10^9 organisms per ml. The suspension was maintained at 20 °C and at 15 min intervals, starting immediately after preparation, 2×10^8 trypanosomes were removed and incubated with 2 CH_{50} units of bovine complement at 37 °C for 30 min. After incubation, the trypanosomes were removed by centrifugation and the complement was added to 10^8 rabbit erythrocytes optimally sensitized with sheep anti rabbit erythrocyte serum. Controls were treated as above using PSG to replace the trypanosomes and were included with each 15 min sample. All assays were incubated at 37 °C for 60 min after which unlysed erythrocytes were removed by centrifugation. The optical densities of the supernatants were measured at 541 nm in a spectrophotometer. Figure 1 shows that the generation of anticomplementary activity is time dependent the lag phase being approximately 3.5 h. Although the complement activating material has not been identified, the kinetics of its production and/or release is similar to that observed in a classical enzyme catalysed substrate conversion curve. In addition, the material which was found in the supernatant of trypanosome suspensions incubated for a minimum of 3 h at 20 °C was stable at 56 °C for 30 min but was destroyed at 100 °C for 15 min. A dose response curve (figure 2) using trypanosomes incubated at 20 °C for 9 h indicates that the number of organisms required to activate 2 CH_{50} units of bovine complement is 2×10^8 for T. congolense and 1.25×10^7 for T. lewisi.

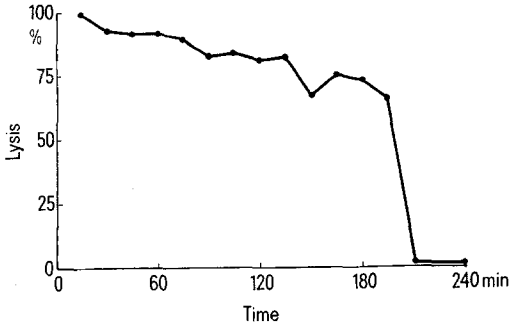

Fig. 1. Generation of complement activating material by 2×10^8 T. congolense with time. The lag time of inhibition of lysis of the indicator system is approximately $3^1/_2$ h for 2 CH_{50} units.

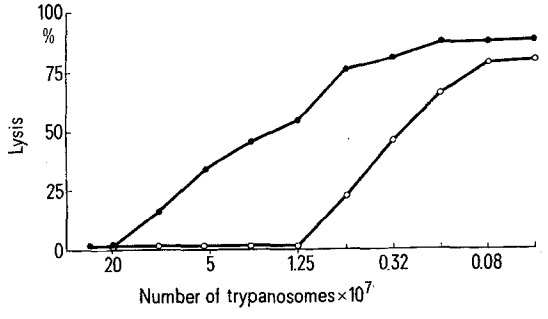

Fig. 2. Dose response curve of activation of 2 CH_{50} unit of bovine complement by various numbers of T. congolense (●–●) and T. lewisi (○–○). The minimum number of T. congolense required to activate this amount of complement is 2×10^8 while 1.25×10^7 T. lewisi were equally effective.

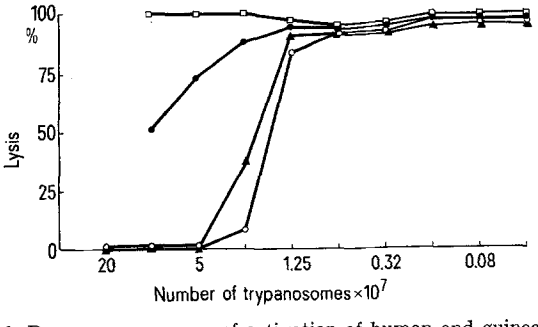

Fig. 3. Dose response curve of activation of human and guinea pig complement by T. congolense and T. lewisi. ●–●, T. congolense + human complement; ○–○, T. congolense + guinea pig complement; ×–×, T. lewisi + guinea pig complement; △–△, T. lewisi + human complement. It should be noted that 5×10^7 T. lewisi was capable of activating 2 CH_{50} units of either human or guinea pig complement, while 10^8 T. congolense could not activate guinea pig complement and reduced the human complement by approximately 1 CH_{50} unit.

Figure 1 shows that the generation of anticomplementary activity is time dependent the lag phase being approximately 3.5 h. Although the complement activating material has not been identified, the kinetics of its production and/or release is similar to that observed in a classical enzyme catalysed substrate conversion curve. In addition, the material which was found in the supernatant of trypanosome suspensions incubated for a minimum of 3 h at 20 °C was stable at 56 °C for 30 min but was destroyed at 100 °C for 15 min. A dose response curve (figure 2) using trypanosomes incubated at 20 °C for 9 h indicates that the number of organisms required to activate 2 CH_{50} units of bovine complement is 2×10^8 for T. congolense and 1.25×10^7 for T. lewisi.

The first component of complement (C1) was isolated from fresh bovine serum by the method of Barta et al.[8]. This material was reconstituted to the original serum volume as was the remainder of the serum (free of C1). The equivalent of 2 CH_{50} units of C1 and of the serum containing C2 to C9 were each reacted with 1.8×10^8 9-h-old trypanosomes for 30 min at 37 °C. After incubation, the trypanosomes were removed by centrifugation and 2 CH_{50} units of C2 to C9 containing serum was added to the C1 previously incubated with the trypanosomes. 2 CH_{50} units of C1 was added to the C2 to C9 serum previously reacted with trypanosomes. Controls treated similarly with PSG but no trypanosomes were included. The indicator system was then added and lysis was measured as above. The results presented in the table clearly indicate that the trypanosomes act at the level of C1 and would therefore be assumed to activate complement by the classical pathway of activation.

T. congolense (10^8) and T. lewisi (2.2×10^8) incubated for 9 h at 20 °C were reacted with 2 CH_{50} units of guinea pig or human complement in an experiment similar to that illustrated in figure 2. These findings are presented in figure 3. It is clear that T. lewisi can activate both human and guinea pig complement in addition to bovine complement, while T. congolense was unable to activate guinea pig complement and could only slightly react with human complement. This observation is difficult to reconcile considering that T. lewisi is a pathogen of rats only, while T. congolense is pathogenic for a wide variety of mammals.

Discussion. The significance of direct activation of complement by trypanosomes is manifold. Thus in microenvironments provided by localization in capillaries the

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	Component(s) $2CH_{50}$	1.8×10^8 T. congolense	Incubation Component(s) $2CH_{50}$	% Lysis
1	C1	+	C2 to C9	0
2	C2 to C9	+	C1	93.9
3	C1	–	C2 to C9	42.7
4	C2 to C9	–	C1	85.2
5	C1	–	–	0
6	C2 to C9	–	–	0

Interaction of T. congolense with functionally pure bovine C1 and C2 to C9. The C1, purified by the method of Barta et al.[8], tended to aggregate and as a result centrifugation after incubation removed some of the C1 needed to react with the C2 to C9 to produce complete lysis of the indicator system. Barta et al.[8] recommend the use of 1.0 M urea to solubilize the C1, but it was felt such treatment would be detrimental to the trypanosomes in tubes 1 and 2.

parasite could avoid immune destruction by activating C1, thereby rendering the lytic process inoperative even in the presence of complement fixing antibody. In addition, trypanosome infected hosts are predisposed to secondary infections, perhaps partly as a result of general destruction of lymphoid tissues, but also as a consequence of diminished lytic processes of infective agents. Deprivation of complement has been shown in experimental animals by Pepys et al.[9–12] (mouse) and White et al.[13] (chicken) to cause disorganization of the architecture of lymphoid tissues. This could also be the case in trypanosomiases in some instances of which marked deterioration of the thymus, lymph nodes, spleen and bone marrow has been reported[6]. Further, the decrease in complement levels in trypanosome infected animals along with the reported increases in serum IgM[3] raises some interesting questions with respect to the influence of this parasite on the homeostatic mechanism governing antibody production. Kohler and Mueller-Eberhard[14] in addition to Glovsky and Fudenberg[15] reported that in some individuals with Waldenstroms macroglobulinemia C1 levels were decreased. In fact, the former authors demonstrated a direct correlation between C1q levels and IgG levels. These findings, along with those of Pepys[9,12] and Nielsen and White[16] who reported homeostatic defects in animals treated with cobra venom factor support premises made with regard the necessity of complement for production of antibody of different immunoglobulin classes.

Finally, it becomes essential to study further the mechanism by which trypanosomes are able to activate complement in order to understand the pathogenesis of this disease. Secondly, it will be necessary to ascertain the impact of this organism on immunoglobulin regulatory mechanisms, as it would appear to be essential knowledge not only to research on protective immunity to the parasite but also for its use as a natural animal model for the future elucidation of the homeostatic mechanism of antibody biosynthesis.

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Some properties of the goat placental lactogen

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Summary. Goat placental lactogen was partially purified from a medium collected after placental tissue incubation. The data obtained by disc electrophoresis and isoelectric focusing experiments, as well as by means of radioreceptor assay methods, provide evidence of the similarity between the goat and ovine placental lactogen.

Recently, ovine and bovine placental lactogens have been purified[2–4] and the existence of goat placental lactogen has been demonstrated[5–7]. In the present paper, some properties of partially purified goat placental lactogen (gPL) are described.

Materials and methods. Placental tissue of Czech white goats at week 17 of pregnancy was used. Explants of about 20 mg were prepared and batches of 10–15 explants were put into Petri dishes containing 4 ml of Waymouth's medium[8]. The medium contained 2 mg/ml glucose, 0.01% penicillin-G, 0.005% streptomycin and 0.002% mycostatin. The tissue explants were incubated at 37 °C in an atmosphere of 95% O_2 and 5% CO_2. The incubation medium was replaced with fresh medium every 24 h. The proteins of the collected medium were gel filtrated on a Sephadex G-25 column, equilibrated with 0.1 M NH_4HCO_3, and freeze-dried. Disc electrophoresis was made on 7.5% polyacrylamide gel (pH 8.9)[9]. Isoelectric focusing was performed on 7.5% polyacrylamide gel columns prepared by photopolymerization[10]. Lactogenic activity of the proteins was estimated using a mouse mammary gland bioassay in vitro[11]. Placental lactogen was monitored by a radioreceptor assay for prolactin (RRA-PRL) using the rabbit mammary gland receptor[12]. Somatotrophin-like activity was estimated by a radioreceptor assay (RRA-GH) employing rabbit liver receptor[13]. The following hormones served as the tracers and the standards: ovine prolactin (30 IU/mg)[14] and bovine growth hormone (1.4 IU/mg: prepared according to the method of Prusík and Braun[15]). The iodinated hormones were prepared by the lactoperoxidase method[16].

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