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Experimental bovine trypanosomiasis

CHANGES IN SERUM IMMUNOGLOBULINS, COMPLEMENT AND COMPLEMENT COMPONENTS IN INFECTED ANIMALS

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Summary. In three calves experimentally infected with *Trypanosoma congolense* the amounts of IgG1 and IgG2 were little changed and similar to those of normal animals. IgM increased in amount early in the infection and the amount of the increase appeared related to the parasite burden. The amounts of IgA and IgE were both much decreased and this also appeared related to the numbers of parasites in the blood.

There was a decrease in the amounts of total haemolytic complement and complement components C1, C1q and C3 in the infected calves. Furthermore the amounts of properdin fluctuated with the cyclical changes in numbers of *T. congolense* parasites in the individual calves. No significant change in the amount of C8 was observed. It is considered that activation of both the alternative and the classical complement pathways occurs in trypanosome infected animals but that neither pathway goes to its terminal stages.

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INTRODUCTION

It is generally accepted that serum IgM levels increase in cattle infected with *Trypanosoma sp.* (Kobayashi & Tizard, 1976; Luckins & Mehlitz, 1976; Luckins, 1974; Clarkson, Penhale, Edwards & Farrell, 1975). While Luckins (1974) and Mehlitz (1976) showed an overall increase in IgG levels of infected cattle, Kobayashi & Tizard (1976) demonstrated a considerable increase in IgG1 levels in *T. congolense* infected cattle, while the IgG2 levels remained relatively constant. The mechanism which is responsible for these increased serum protein levels is not understood; however, it has been postulated by Hudson, Byner, Freeman & Terry (1976) to be a result of polyclonal B cell stimulation by the trypanosome in a series of experiments in mice. If such were the case, increased levels of other immunoglobulins, such as IgA and IgE might also be expected.

We report on the serum IgM, IgG1, IgG2, IgA and IgE levels of cattle experimentally infected with *T. congolense*.

We also show that complement is activated by the classical sequence, and that properdin is activated in infected calves. However, there was no change in the amount of serum C8, and it is concluded that the terminal complement components are not utilized.

MATERIALS AND METHODS

Animals

Five Holstein bull calves were used. Three of these animals were infected by intravenous injection with $5\cdot5\times10^8$ *Trypanosoma congolense* (nos 422, 423 and 424) and the remaining two were maintained as controls (nos 150 and 152). All animals were bled three times a week and the sera obtained were stored at $-70°$ until assayed. The infected and control calves were housed under similar environmental conditions.

Assay of immunoglobulins and complement components

IgG1. This was prepared by gel filtration and ion exchange chromatography of whey and used to immunize rabbits. The resulting antisera contained anti IgG1 and IgG2. The anti-IgG2 was removed by IgG2, prepared as below, and polymerized with glutaraldehyde (Avrameas & Ternynck, 1969) to yield a monospecific antiserum.

IgG2. IgG2 was prepared by ion exchange chromatography by the method of Duncan, Wilkie, Hiestand & Winter (1972). Antiserum prepared in rabbits to this fraction was absorbed with polymerized IgG1.

IgM. Rabbit anti bovine IgM was prepared by the method of Nielsen (1976).

IgA. This was obtained from nasal washing purified by ion exchange chromatography by the method of Duncan *et al.* (1972), and the rabbit antiserum to this antigen was purified by absorption with polymerized IgG1 and IgG2 to obtain a monospecific antiserum.

Reaginic IgE-like globulin was separated by the method of Nielsen & Wilkie (1977) and used to prepare a rabbit anti-IgE antibody which did not react with other bovine immunoglobulins when tested by a radio-immunodiffusion assay (Nielsen, 1977) but did react with a reagin-rich globulin fraction prepared according to Hammer, Kickhofen, & Schmid (1971).

Complement C1 was prepared by the method of Barta, Nelson & Kuo (1976), and rabbit antiserum to this antigen was purified by absorption with IgG2 (Duncan *et al.*, 1972) and a Sephadex G200 protein fraction, all polymerized with glutaraldehyde (Avrameas & Ternynck, 1969).

Complement C1q was prepared by the method of Sledge & Bing (1973).

Rabbit antibovine C3 was purchased from Cappel Laboratories, Inc., Cochranville, Pa., U.S.A. Lot No. 9931 and the contaminating anti-IgM antibodies were removed by absorption.

Bovine C8 was isolated from the serum fraction remaining after the removal of C1 as described previously. The protein separated by chromatography (see Barta *et al.*, 1976) was used to immunize rabbits, and the resulting anti-C8 was purified by repeated absorption with IgG2 polymerized by glutaraldehyde.

An antibovine properdin was prepared by the method described for separation of human properdin (Pensky, Hinz, Todd, Wedgewood, Boyer & Lepow, 1968).

The amounts of all globulins were determined against standards of purified fractions by the method of Mancini, Carbonara & Heremans (1965) with the exception of the test for IgE and properdin. As no pure fraction of IgE was available as a standard, the amounts of IgE were compared to those of normal animals by comparing the precipitation ring diameter.

The antiproperdin serum did not give a visible precipitate by gel diffusion so the globulin fraction containing it was radiolabelled and used in the radio-immunodiffusion assay of Nielsen (1977).

Total haemolytic complement levels were determined on all serum samples using rabbit erythrocytes optimally sensitized by sheep haemolysin. Dilutions of the serum samples were incubated with the indicator system at 37° for 1 h after which any unlysed cells were removed by centrifugation (500 *g* for 5 min). Released haemoglobulin was measured at 541 nm in a spectrophotometer. CH50 units were calculated using the von Krogh equation (Kabat & Mayer, 1961).

RESULTS

Immunoglobulins

The parasite counts of the infected animals (Fig. 1) showed one calf (no. 422) was heavily parasitized, one (423) had a moderate parasitaemia, and the third calf (424) was only lightly infected though all three animals received the same inoculum of $5\cdot5\times10^8$ viable trypanosomes.

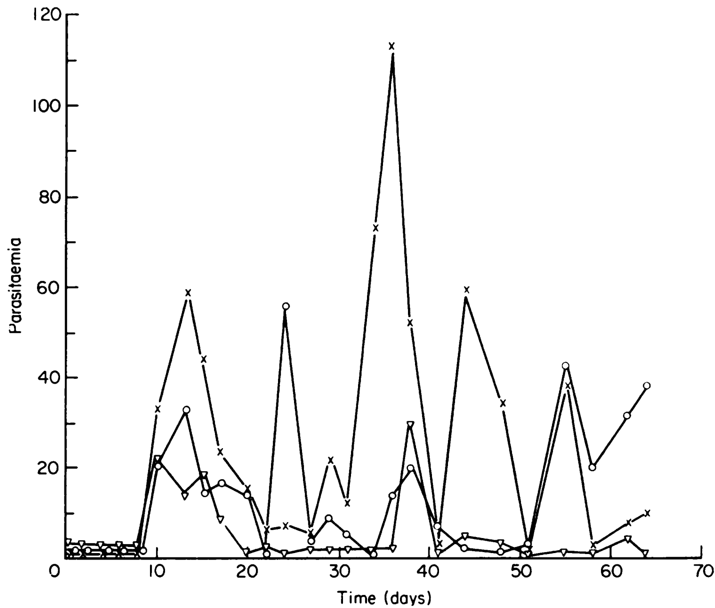

Figure 1. Time course of parasitaemia in three calves infected with $5{\cdot}5 \times 10^8$ *Trypanosoma congolense*. The parasite count represents the number of trypanosomes, as judged by their motility, counted in twenty-five fields of a wet smear of whole blood magnified ×400. (×) calf no. 422; (▽) calf no. 423; (○) calf no. 424.

There was no significant increase in the amount of IgG1 above normal though some fluctuation in amount was observed (Fig. 2). The amounts of IgG2, though fluctuating during the period of the experiment, were no different from the control animals. Calves no. 422 and 423 which started the test at a higher than normal concentration of IgG2, continued to have this slightly raised amount.

The amount of IgM was increased 10 days after infection and continued to be increased throughout

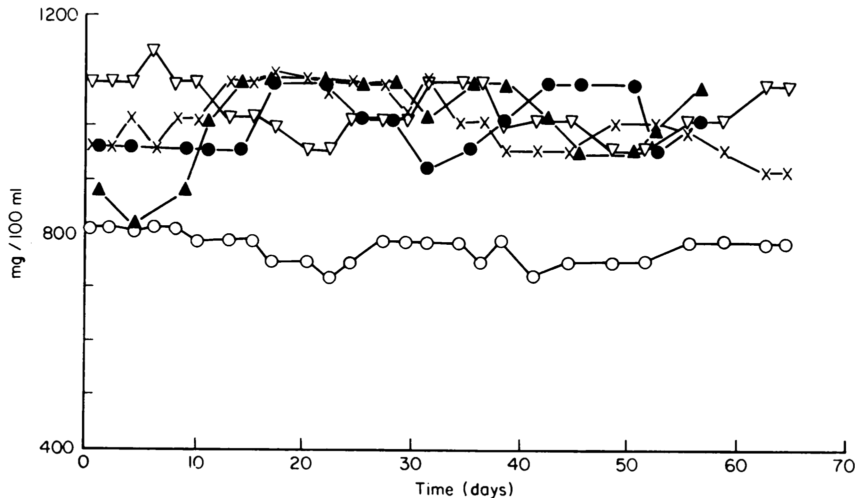

Figure 2. Serum IgG1 levels of *Trypanosoma congolense* infected calves (×) calf no. 422; (▽) calf no. 423; (○) calf no. 424 and normal calves (▲) calf no. 150; (●) calf no. 152 in mg/100 ml of serum over the time course of the experiment.

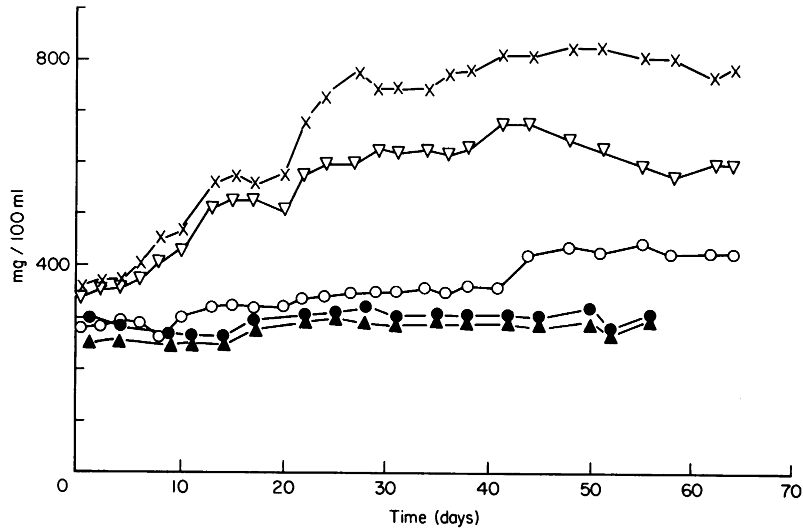

Figure 3. Serum IgM levels of *Trypanosoma congolense* infected calves (×) calf no. 422; (▽) calf no. 423; (○) calf no. 424 and normal calves (▲) calf no. 150; (●) calf no. 152 in mg/100 ml of serum over the time course of the experiment.

the period, though reaching a plateau in amount at about 30 days post infection (Fig. 3).

The amounts of IgA in the serum of the control calves (no. 150 and 152) were low at the start of the test but increased steadily to the normal range about day 30 (Fig. 4) though the infected animals had a gradual but dramatic decrease to one quarter or one fifth of the concentration at the start by 25 days post inoculation.

The amounts of serum IgE in the infected animals began to decrease about the 10th day post inoculation (Fig. 5) in contrast to the serum IgA amounts

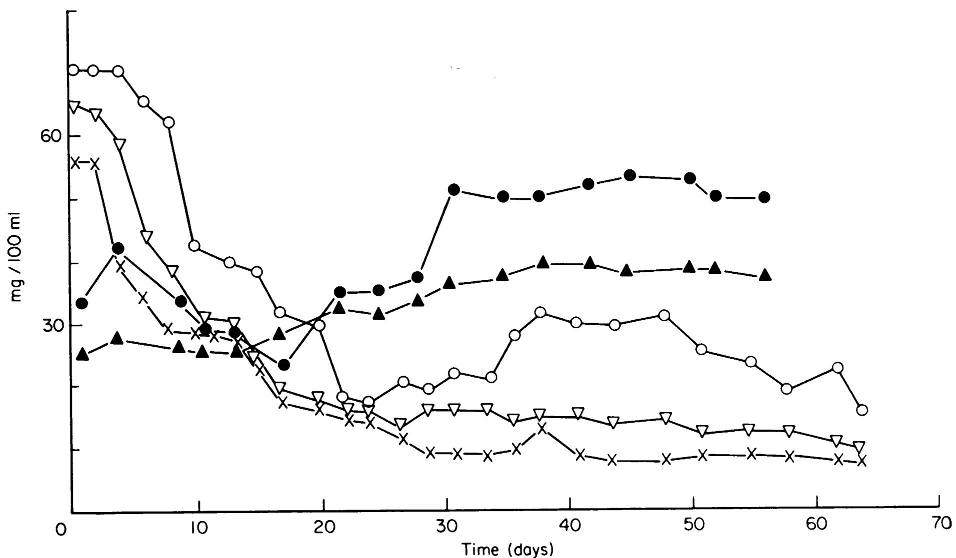

Figure 4. Serum IgA levels of *Trypanosoma congolense* infected calves (×) calf no. 422; (▽) calf no. 423; (○) calf no. 424 and normal calves (▲) calf no. 150; (●) calf no. 152 in mg/100 ml of serum over the time course of the experiment.

which began to decrease at 2 to 4 days after infection.

It is particularly interesting that changes in serum IgM and IgE levels start at about the time the first parasitaemia peak begins while the decrease in serum IgA levels precede any observable blood parasitaemia. In addition, the magnitude of the change in serum IgM, IgA and IgE seems to be directly in proportion to the severity of the parasitaemia. Thus, animal no. 422 which was consistently the most heavily parasitized also showed the greatest changes in its serum immunoglobulin levels. Animals no. 423 and 424 which had fewer blood parasites also had proportionately smaller changes in their IgM, IgA and IgE levels.

Complement

The haemolytic complement levels (Fig. 6) of the control calves was maintained at about 14 CH_{50} units/ml, but the infected calves all showed a decrease starting about 8 days after infection. Infected calf no. 422 had the greatest decrease in haemolytic complement and was the most severely parasitized (Fig. 1).

Infected calves 423 and 424, though less severely parasitized also showed a considerable decrease in haemolytic complement. The onset of the initial decrease in complement corresponded with the first increase in number of parasites in the blood, but thereafter the fluctuations in amounts of complement bore no relationship to the fluctuation in numbers of parasites (Fig. 1).

The amounts of C1 in the sera of normal calves fluctuated by about 10%, whereas there were decreases of 75%, 60% and 50% in the infected calves (Fig. 7). The decrease in C1 occurred before the infection was apparent and remained low thereafter.

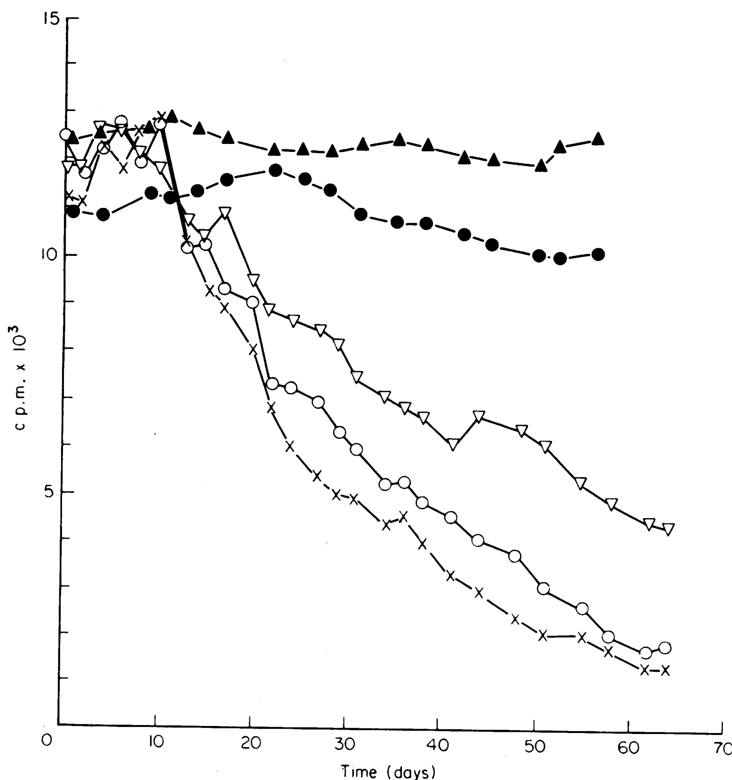

Figure 5. Serum IgE levels of *Trypanosoma congolense* infected calves (×) calf no. 422; (▽) calf no. 423; (○) calf no. 424 and normal calves (▲) calf no. 150; (●) calf no. 152. The ordinate (c.p.m. $\times 10^3$) is indicative of the amount of [^{125}I] labelled anti-bovine reagin retained as immune complexes in a radioimmunodiffusion assay. The abscissa represents the time course of the experiment.

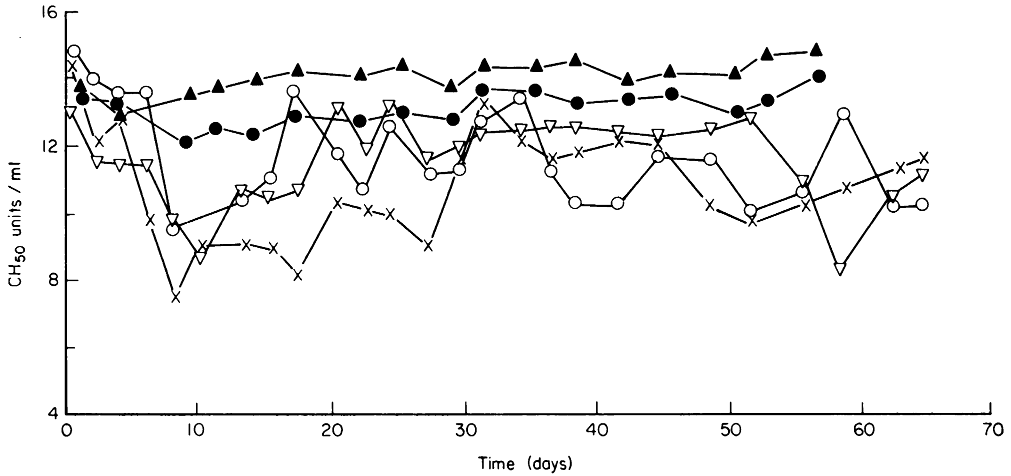

Figure 6. Time course plot of serum haemolytic complement levels (CH_{50} units /ml) in calves infected with *T. congolense* (×) calf no. 422; (▽) calf no. 423; (○) calf no. 424 and normal calves (▲) calf no. 150; (●) calf no. 152.

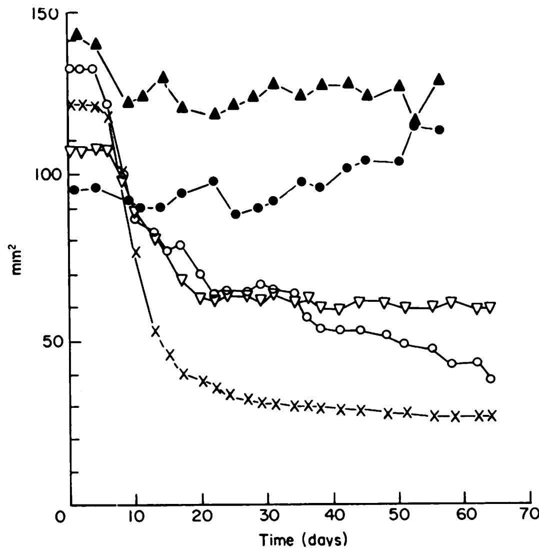

Figure 7. Time course plot of serum levels of the first component of complement (C1) measured as precipitin rings (mm^2) in radial immunodiffusion assays in calves infected with *T. congolense* (×) calf no. 422; (▽) calf no. 423; (○) calf no. 424 and in normal calves (▲) calf no. 150; (●) calf no. 152.

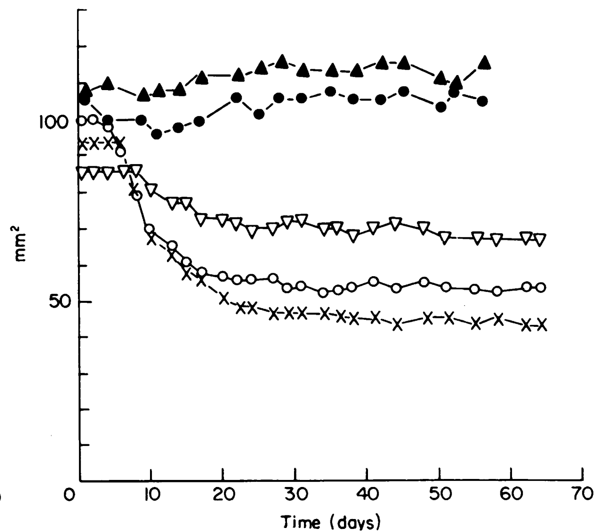

Figure 8. Time course plot of serum levels of C1q subcomponent of the first component of complement measured as precipitin rings (mm^2) in radial immunodiffusion assays. (×) calves no. 422; (▽) calf no. 423; (○) calf no. 424 were infected with *T. congolense* while animal nos 150 (▲) and 152 (●) were normal.

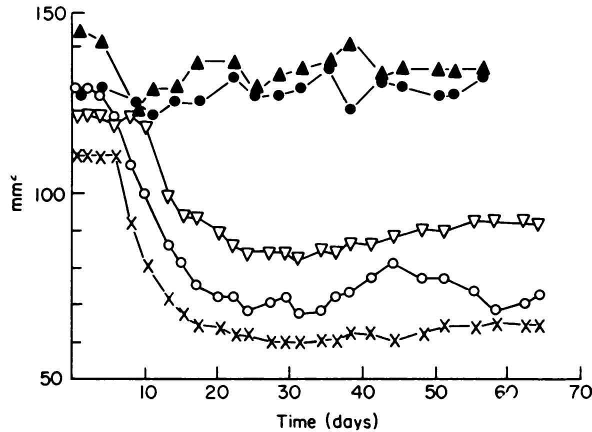

Figure 9. Time course plot of serum levels of the third component of complement (C3) measured as precipitin rings (mm²) in radial immunodiffusion assays. Calves no. 422 (×); 423 (▽); and 424 (○) were infected with *T. congolense* while animal nos 150 (▲) and 152 (●) were normal.

The rate of decrease of C1q (Fig. 8) and C3 (Fig. 9) resembled that of C1.

Infection did not change the amounts of C8 during the period of the study. However, the amounts of properdin (Fig. 10) appeared to decrease and increase with the fluctuations in numbers of blood parasites though occurring one to two days after the changes in numbers of parasites.

DISCUSSION

Most reports in the literature dealing with immunoglobulin levels of trypanosome infected cattle have showed no correlation between immunoglobulin levels and parasitaemia because of averaging of results obtained at each individual sampling. However, if each animal is considered as a single entity, it would appear that the severity of infection correlates well with overall serum immunoglobulin A, M and E levels as reported in this study.

In our study which lasted for about 65 days, no apparent differences were seen in the serum IgG1 and IgG2 levels. While these findings are not in keeping with those of Kobayashi & Tizard (1976) who showed that serum IgG1 levels were elevated

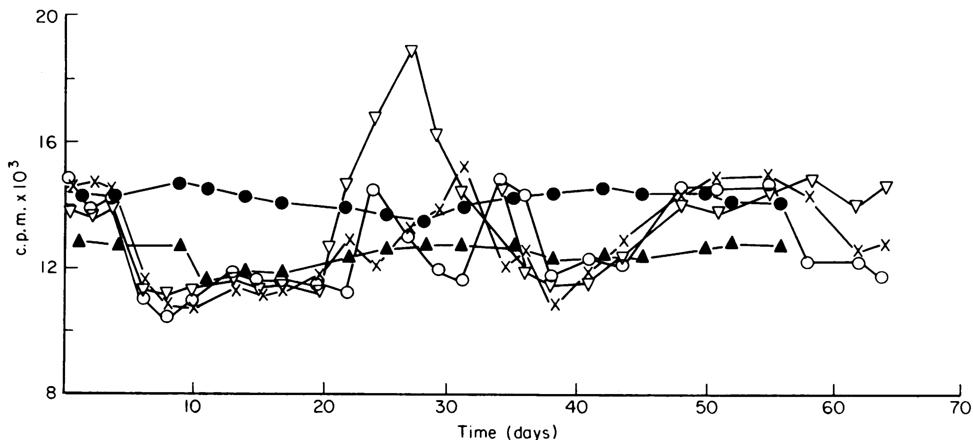

Figure 10. Time course plot of serum levels of properdin measured in radio immunodiffusion assays (c.p.m.). Calves no. 422 (×); 423 (▽) and 424 (○) were infected with *T. congolense* while animal nos 150 (▲) and 152 (●) were normal.

to 2·5 times the preinfection levels at 7 weeks postinfection, Clarkson *et al.* (1975) have reported no changes in IgG for the first 65 days of infection with *T. vivax* in some animals. Together with other findings by Clarkson & Penhale (1973) and with those of Luckins (1972) all of which suggest 1·5 to two fold increases in IgG 2–4 weeks postinfection, a rather inconstant picture emerges. Thus, it would appear that serum IgG levels may or may not rise in trypanosome infected animals perhaps depending on the breed of animals used, their state of health through the course of infection and environmental conditions.

There is general agreement in published reports with regard to elevated IgM levels of trypanosome infected cattle. Thus, Kobayashi & Tizard (1976) demonstrated six to twenty-four times preinfection IgM levels at 2–3 weeks postinfection. Similar findings have been recorded by Clarkson & Penhale (1973) for *T. vivax* infected cattle, by Luckins (1975) for cattle naturally infected with *T. congolenses* and by Van Miervenne, Moors & Janssens (1972) for *T. brucei* infected cattle. While our cattle did have increased IgM levels, these were not as dramatic as those reported elsewhere, perhaps as our results indicate, depending on the parasite burden of the individual animal.

Serum IgA and IgE levels were quite diminished in trypanosome infected cattle, again apparently depending on the parasite count of the individual animals. The significance of these findings is not clear but since secondary infections are a problem in trypanosome infected animals (Losos & Ikede, 1972), perhaps it would be worthwhile investigating if the levels of these immunoglobulins are also reduced at external mucosal surfaces where they are at least partly responsible for protection against invading pathogens.

In the present study it has also been clearly demonstrated that, in trypanosome infected animals, consumption of complement takes place. Thus the early complement components (C1, C1q and C3) as well as total haemolytic complement levels were decreased throughout most of the infection during the two months of this investigation. The changes in complement levels commenced at or slightly before trypanosomes were detectable in blood smears. While minor fluctuations were in evidence in the serum levels of early complement components of normal animals, none of these changes assumed the magnitude apparent in infected cattle. While trypanosome infection had no apparent effect on the serum C8 levels, a decrease in properdin levels just prior to peaks in parasitaemia were noted. Such fluctuations were not observed in the two normal animals.

Complement activation in trypanosomiasis has been reported previously. Thus in man, Mulligan (1970) reported decreased complement levels and Greenwood & Whittle (1976) reported low levels of C3, C4 and factor B in the sera of patients with Gambian trypanosomiasis. Further, Kierszenbaum & Weinman (1977) reported antibody independent activation of human complement by *T. cyclops*. This activation was thought to be of the alternative complement pathway. In addition, Nagle, Ward, Lindsley, Sadun, Johnson, Berkow & Hildebrandt, (1974) showed that monkeys experimentally infected with *T. rhodesiense* were hypocomplementaemic in conjunction with proliferative glomerulonephritis and had decreased serum C3, but not C4 levels. Similarly, Jarvinen & Dalmasso (1976) showed that in *T. lewisi* infected rats parasite counts correlated with serum C3 levels, but not with total serum complement levels. They concluded that *T. lewisi* infection resulted in activation of the classical complement pathway. Kobayashi & Tizard (1976) found that anaemia in cattle infected with *T. congolense* coincided with a drop in parasitaemia, the appearance of complement-fixing antibody and a progressive decrease in serum C3 levels.

These reports from the literature lead to a rather confusing picture as to the mechanism of the complement activation by trypanosomes. However, considering previous reports by Nielsen & Sheppard (1977) and Nielsen & Wilkie (1977) along with the above findings, it would appear that both the classical and the alternative pathways of complement activation are involved. Thus *in vitro* trypanosomes in the absence of antibody can activate C1 (Nielsen & Sheppard, 1977) and C3 Kierszenbaum & Weinman, 1977). Similarly, *in vivo* both the alternative pathway (Nagle *et al.*, 1974) and the classical pathway were activated (Jarviven & Dalmasso, 1976) presumably in the presence of antibody. The results reported in this communication would appear to support the *in vivo* activation of both C1 and C3 on a continuous basis and probably therefore in the absence of antibody, being apparently unaffected by the rise and fall in immune-complex. levels occurring as a result of antigenic variation. In addition, properdin appeared to be activated in

relation to the parasite count while C8 was apparently not involved. These findings would tend to suggest that immune complex activation was not involved, but rather implied direct activation by substances produced by the microorganisms.

Activation of complement has several important implications not only in the pathogenesis of trypanosomiasis but also in terms of 'satellite' pathology. Experimental decomplementation has been shown to have profound effects on the immune response (reviewed by Pepys, 1976). Decreased complement levels in trypanosomiasis may therefore be involved not only in the changes in immunoglobulin levels (Kobayashi & Tizard, 1976) but also in the immunosuppression phenomenon (Hudson *et al.*, 1976). Another interesting implication of trypanosomiasis is the reported relationship between *T. theileri* infections of cattle and lymphocytosis (Cross, Smith & Redman 1971; Cross *et al.*, 1968). Although no correlation between the presence of C-type virus particles and *T. theileri* infection of cattle was shown by Mammerickx & Dekegel (1975), sixteen of their cattle were found positive for lymphocytosis, C-type particles and trypanosomiasis while nine were positive as above but negative for *T. theileri*. Thus *T. theileri* infection of cattle, which is quite a common phenomenon at least in Ontario (Woo, Soltys & Gillick, 1970), if indeed *T. theileri* does activate complement in the host, may cause a drop in complement levels sufficient to predispose such animals not only to aberrations in their immune response but also to the expression of secondary infections. Obviously much more research is needed in this area, particularly with cattle with genetically low complement levels.

ACKNOWLEDGMENTS

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