Acta vet. scand. 1985, *26*, 191—204.

From the Danish Bilharziasis Laboratory and Institute of Veterinary Microbiology and Hygiene, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

SUPPRESSION OF BABESIA MICROTI INFECTION IN MICE CONCURRENTLY INFECTED WITH SCHISTOSOMA MANSONI

By

Benjamin Fagbemi, Niels Ørnbjerg Christensen and *Peter Nansen*

FAGBEMI, BENJAMIN, NIELS ØRNBJERG CHRISTENSEN and PETER NANSEN: *Suppression of Babesia microti infection in mice concurrently infected with Schistosoma mansoni.* Acta vet. scand. 1985, *26*, 191—204. — Blood cell parasitaemias of Babesai microti and associated haematological changes were studied in mice concurrently infected with Schistosoma mansoni and in controls with no schistosome infections. In comparison to the controls B. microti parasitaemias were suppressed markedly in mice harbouring 6 and 8 weeks old patent S. mansoni infections at the time of infection with B. microti. The suppression was accompanied by an alleviation/annulment of the B. microti induced reductions in haemoglobin and haematocrit levels and in its erythropoetic stimulation. The suppressive effect of patent S. mansoni infections on B. microti is suggested to be at least partly attributable to non-specific immunological factors although the altered erythrocyte kinetic state induced by the schistosoma infection in combination with the preference of B. microti for mature (old) erythrocytes might also play a role in the suppression. In mice harbouring 2 weeks old prepatent S. mansoni infections B. microti was not suppressed.

rodent model; double infection; parasitaemia; haematological parameters; altered erythrocyte kinetic state; immunological factors.

Infections of domestic stock animals and man with more than one parasite species at the same time is the rule rather than the exception (*Willett* 1972, *Buck et al.* 1978a, b) and the possibility that this may give rise to heterologous interactions has stimulated an increasing interest in experimental studies designed to analyse various aspects of multiple parasitism in the same definitive host. In fact, concurrent experimental infections with some helminths

and protozoans have shown that a primary helminth infection may augment a superimposed protozoan infection (*Kloetzel et al.* 1973, *Yusuf et al.* 1980) and the reverse situation, i.e. the suppression by a helminth infection of a superimposed protozoan infection is also well documented (*Ngwenya* 1982, *Bailenger & Guy* 1982, *Lwin et al.* 1982). However, the accompanying effects of such interactions on the associated disease patterns have until now not been the subject of much study.

The experiments described in the present paper were conducted to elucidate possible effects of primary prepatent and patent Schistosoma mansoni infections on the course of a superimposed Babesia microti infection in mice.

MATERIALS AND METHODS

Mouse and parasite material

Outbred, albino, female mice obtained from the State Veterinary Serum Laboratory, Copenhagen, Denmark weighing 25—30 g at the beginning of the experiments were used. Cercariae of S. mansoni (Puerto Rican strain) were obtained from Biomphalaria glabrata snails. B. microti (King's strain) was originally obtained from the Centre for Tropical Veterinary Medicine, University of Edinburgh, Scotland and the protozoan was maintained by needle passage in mice.

Infection procedure and measurements of parasitological parameters

Infection of mice with S. mansoni cercariae (120 cercariae/mouse) was made using the ring method (*Smithers & Terry* 1965). Adult schistosomes were recovered by perfusion (*Smithers & Terry* 1965) and the presence of schistosome eggs in faeces was confirmed using the Bell method (*Bell* 1963).

B. microti containing blood for mouse infections was obtained from the cut tail tip of donor mice and the parasites were injected intraperitoneally using 10^6 organisms per mouse after dilution of blood with phosphate buffered saline. B. microti parasitaemia (percentage parasitized red blood cells) was monitored by examining 1000 red cells in Giemsa-stained thin blood smears and the frequency of multiple B. microti per erythrocyte was assessed based on the number of piroplasms in each of 100 parasitized erythrocytes.

Haematological parameters

Blood samples were obtained from the cut tail tip of the mice and haemoglobin concentrations were determined by the cyanomethaemoglobin method. Reticulocyte counts were determined by the method of *King* (1973) and haematocrit values were measured by conventional techniques.

Experimental plans

Three experiments (Exp. 1, 2 and 3) were conducted in which B. microti was given to mice harbouring 2, 6 and 8 weeks old primary S. mansoni infections, respectively, and to corresponding non-infected controls. Each experiment also included a third group infected with S. mansoni only and a fourth group of parasite-free mice. The number of mice in each of all groups was 10. In each experiment B. microti parasitaemia and haematological parameters were determined once weekly for 7 weeks following B. microti infection. Besides, in Exp. 2 and 3 the number of piroplasms per parasitized erythrocyte was determined. At the end of the experiment the size of the established schistosome infection was determined and prior to the start of each experiment involving mice harbouring primary patent schistosome infections at the time of infection with B. microti (Exp. 2 and 3) the establishment of the schistosome infection was confirmed by the demonstration of schistosome eggs in faeces.

The Student's t-test was used to assess significance of differences between mean values and $P < 0.05$ was considered significant.

RESULTS

The results obtained from Exp. 1 (Figs. 1 and 4A) show that a 2 weeks old prepatent S. mansoni infection had no effects on the course of a superimposed B. microti infection. The reticulocyte counts and the reductions in haemoglobin and haematocrit levels in the group of mice concurrently infected with S. mansoni and B. microti were as could be explained from the effects of each of the parasites acting separately except at week 6 where the haematocrit value of the double infected group was inexplicably significantly higher than in the group of mice infected with S. mansoni only. The number of worm pairs recovered at the end of the experiment in the S. mansoni infected group only and in

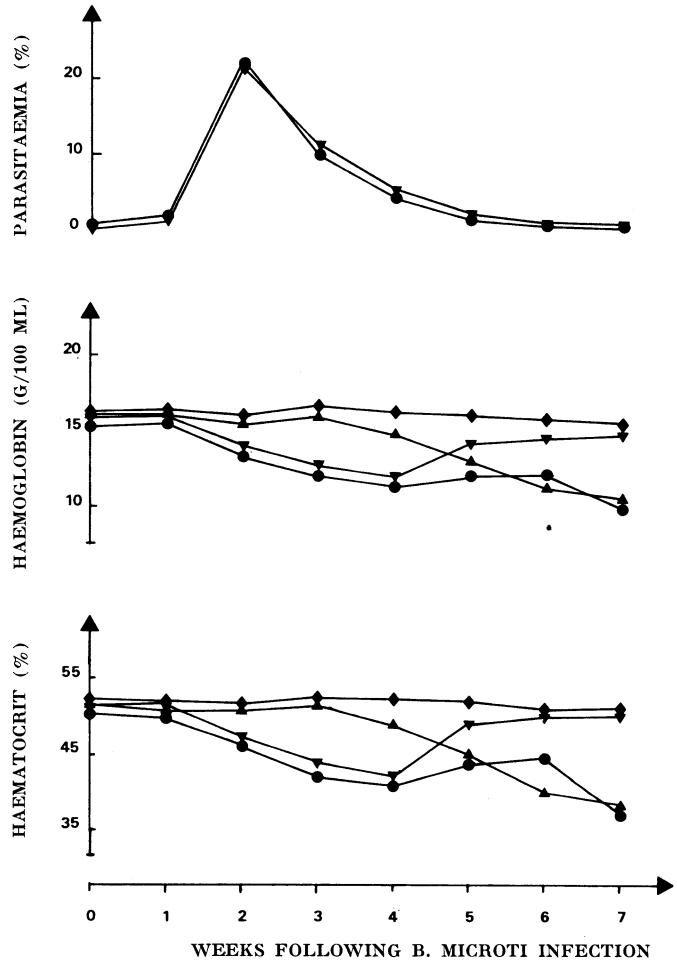

Figure 1. Babesia microti parasitaemia and haematological parameters (haemoglobin, haematocrit) in mice harbouring 2 weeks old Schistosoma mansoni infections at the time of infection with B. microti and in mice infected with B. microti or S. mansoni only (Exp. 1). (▼) B. microti only; (●) S. mansoni plus B. microti; (▲) S. mansoni only; (◆) Parasite-free.

the S. mansoni plus B. microti infected group was 22 ± 4 and 25 ± 6, respectively.

The results obtained from Exp. 2 (Fig. 2, 4B and 5) show that a primary 6 weeks old newly patent S. mansoni infection suppressed a superimposed B. microti infection. The B. microti parasitaemia in the schistosome infected group was thus sup-

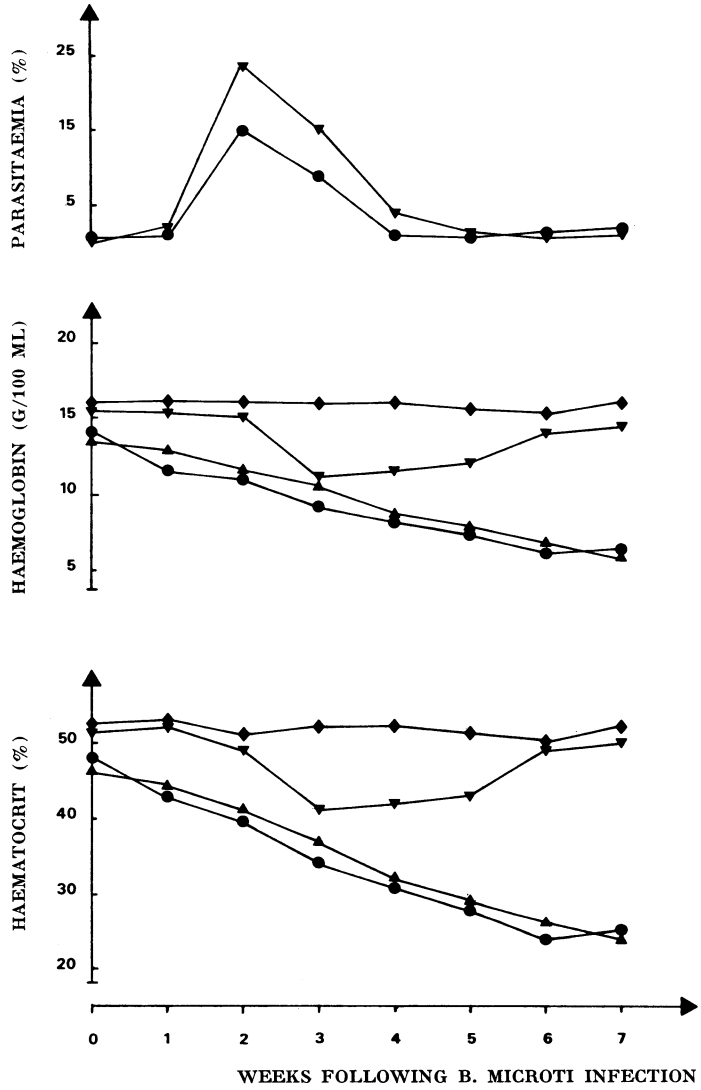

Figure 2. Babesia microti parasitaemia and haematological parameters (haemoglobin, haematocrit) in mice harbouring 6 weeks old Schistosoma mansoni infections at the time of infection with B. microti and in mice infected with B. microti or S. mansoni only (Exp. 2). (▼) B. microti only; (●) S. mansoni plus B. microti; (▲) S. mansoni only; (◆) Parasite-free.

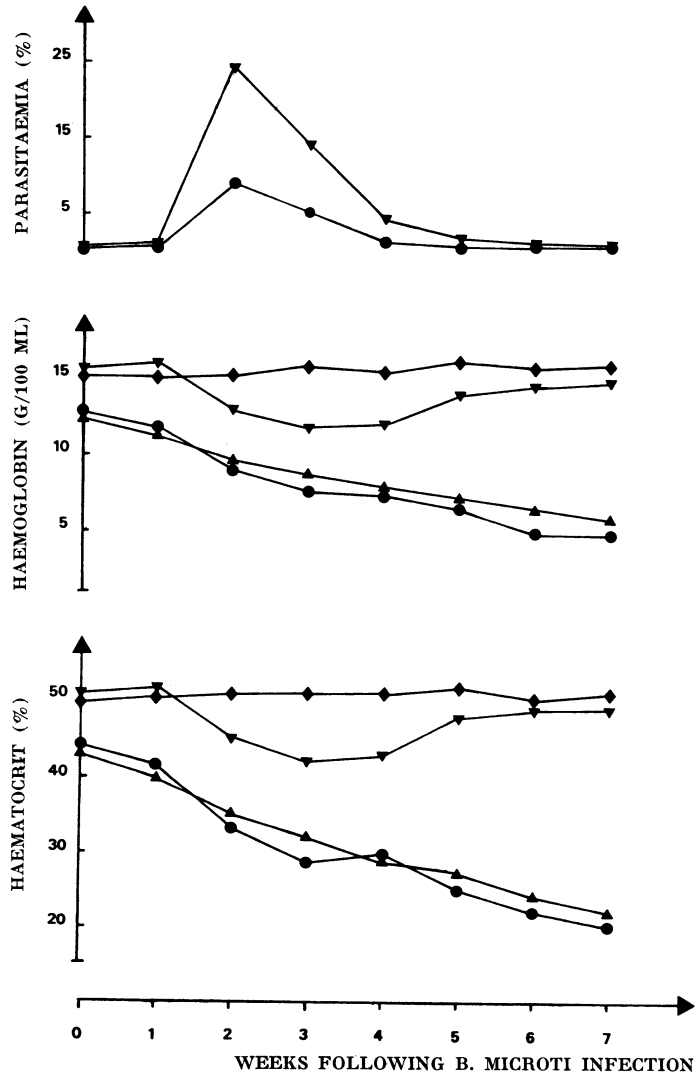

Figure 3. Babesia microti parasitaemia and haematological parameters (haemoglobin, haematocrit) in mice harbouring 8 weeks old Schistosoma mansoni infections at the time of infection with B. microti and in mice infected with B. microti or S. mansoni only (Exp. 3). (▼) B. microti only; (●) S. mansoni plus B. microti; (▲) S. mansoni only; (◆) Parasite-free.

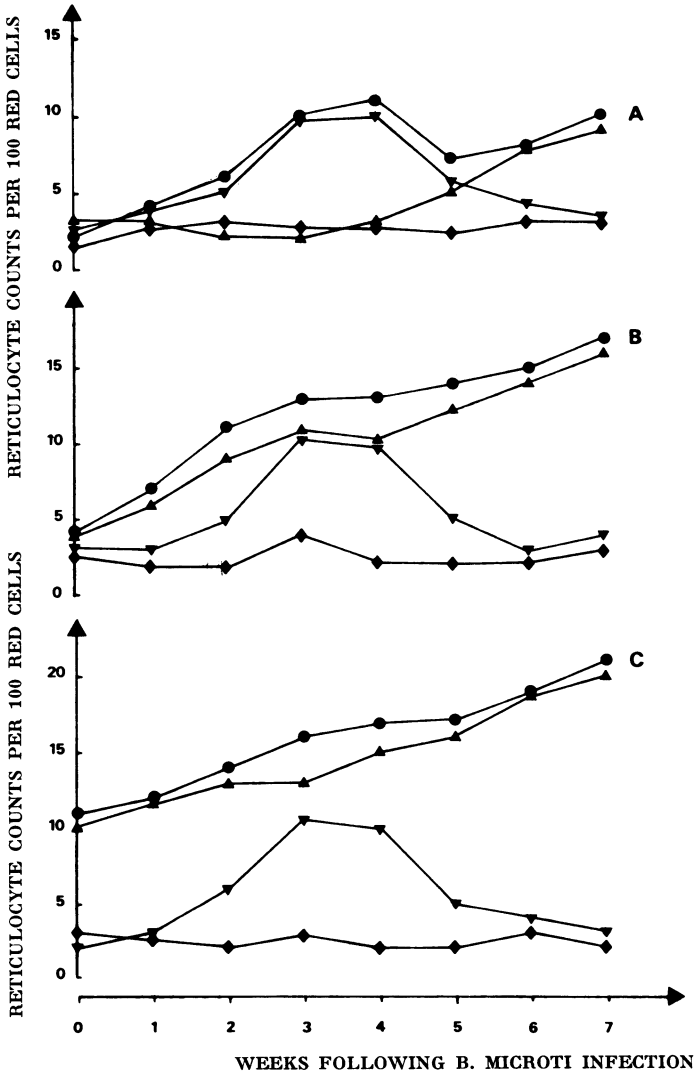

Figure 4. Reticulocyte counts per 100 red cells in mice harbouring 2 (A, Exp. 1), 6 (B, Exp. 2) and 8 (C, Exp. 3) weeks old Schistosoma mansoni infections at the time of infection with B. microti and in mice infected with S. mansoni or B. microti only. (▼) B. microti only; (●) S. mansoni plus B. microti; (▲) S. mansoni only; (◆) Parasite-free.

pressed to a statistically significant level weeks 2, 3 and 4 following infection as compared with that of the protozoan monoinfection. After week 4 the difference in parasitaemia between the 2 groups diminished and the level of parasitaemia became low (Fig. 2). Besides, the frequency of multiple B. microti infections per erythrocyte week 3 and 4 was markedly lower in the double infected group than in the group infected with B. microti only (Fig. 5). From Fig. 2 it will be seen that S. mansoni alone induced a progressive anaemia and that B. microti alone induced a transient anaemia reaching a statistically significant level weeks 3, 4 and 5 following infection as shown by the reductions in the haemoglobin and haematocrit values. However, it will also appear that the haemoglobin and haematocrit values were comparable throughout the experimental period in the S. mansoni infected group only and in the S. mansoni plus B. microti infected group, i.e. a superimposed B. microti infection on an already established 6 weeks old patent S. mansoni infection did not further reduce the level of anaemia developed. The reticulocyte counts reflecting the rate of the erythropoesis are illustrated in Fig. 4B and it will appear that the S. mansoni infection induced a progressive reticulocytosis reaching a statistically significant level from week 2 and onwards and that B. microti alone induced a statistically significant increase in reticulocyte counts weeks 3 and 4 after infection. However, although the reticulocyte counts in the S. mansoni plus B. microti infected group were consistently higher than in the group infected with S. mansoni only the differences never reached a statistically significant level, i.e. a superimposed B. microti infection on an already established 6 weeks old patent S. mansoni infection did not significantly raise the rate of the erythropoesis. The number of worm pairs recovered at the end of the experiment in the S. mansoni infected group only and in the S. mansoni plus B. microti infected group was 27 ± 6 and 24 ± 6, respectively.

The results obtained from Exp. 3 (Fig. 3, 4C and 6) using mice harbouring primary 8 weeks old S. mansoni infections at the time of infection with B. microti were comparable with those obtained in mice harbouring 6 weeks old S. mansoni infections (Exp. 2), but the S. mansoni induced anaemia and associated reticulocytosis and the level of suppression of B. microti as shown by the B. microti parasitaemia (Fig. 3) and by the frequency of multiple B. microti infections per erythrocyte (Fig. 6) were much

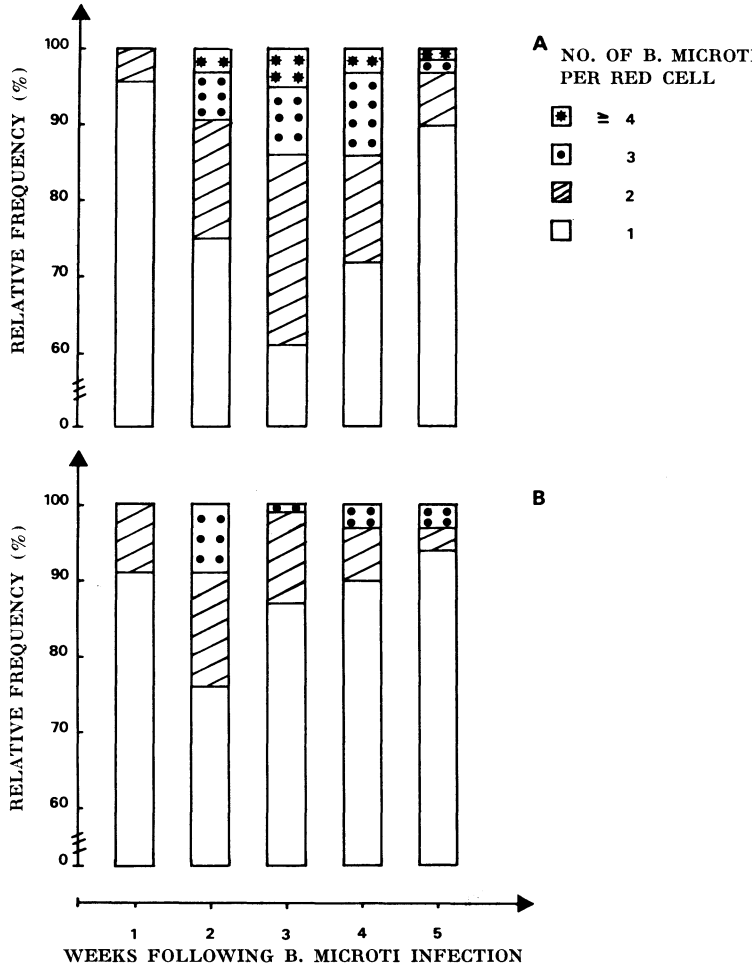

Figure 5. Relative frequency (%) of number of Babesia microti per parasitized red blood cell in mice harbouring 6 weeks old Schistosoma mansoni infections at the time of infection with B. microti and in mice infected with B. microti only (Exp. 2). A: B microti only; B: S. mansoni plus B. microti.

more marked. This suppression of B. microti was also illustrated by the fact that the superimposed B. microti infection on the already established S. mansoni infection did not give rise to any further reduction in the haemoglobin and haematocrit values (Fig. 3) and by the fact that no statistically significant differences could be demonstrated between reticulocyte counts of the S.

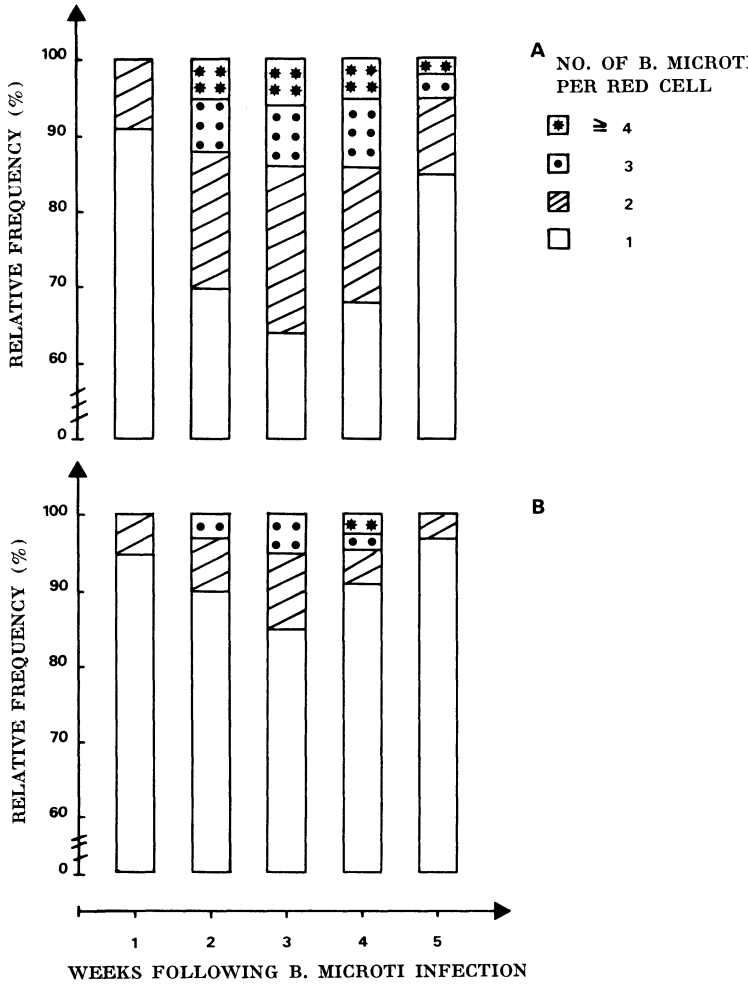

Figure 6. Relative frequency (%) of number of Babesia microti per parasitized red blood cell in mice harbouring 8 weeks old Schistosoma mansoni infections at the time of infection with B. microti and in mice infected with B. microti only (Exp. 3). A: B. microti only, B: S. mansoni plus B. microti.

mansoni and the S. mansoni plus B. microti infected groups of mice (Fig. 4C). The number of worm pairs recovered at the end of the experiment in the S. mansoni infected group only and in the S. mansoni plus B. microti infected group was 18 ± 6 and 15 ± 4, respectively.

DISCUSSION

The results from the present study show that primary patent 6 and 8 weeks old S. mansoni infections in mice may suppress a superimposed B. microti infection and this finding is in line with the demonstration by *Fagbemi et al.* (1985) of a Fasciola hepatica induced suppression in mice of B. microti. The S. mansoni induced suppression of B. microti results in a reduction in both the percentage of erythrocytes parasitized and in the frequency of multiple B. microti infections per parasitized red cell. A corresponding alleviation/annulment of the B. microti induced reductions in haemoglobin and haematocrit and of the contribution of B. microti to the rate of the erythropoesis in S. mansoni infected mice was likewise demonstrated.

The suppression of Plasmodium chabaudi in mice harbouring primary patent S. mansoni infections (*Lwin et al.* 1982) and of B. microti in F. hepatica infected mice (*Fagbemi et al.* 1985) was tentatively suggested to be at least partly due to the altered red cell age profile (i.e. an increased ratio of young to mature red cells) in combination with the reported preference of P. chabaudi and many Babesia species for mature red cells (*Simons* 1939, *Landsberg & Eskridge* 1940, *McHardy* 1973, *Hussein* 1976, *Lwin et al.* 1982). In the present study a marked change in the erythrocyte kinetics and age profile was observed in the mice harbouring patent S. mansoni infections at the time of infection with B. microti and the possibility exists that the suppression may be at least partly due to the blood environment being less favourable for the survival and reproduction of the protozoan. However, preliminary studies by *Andersen et al.* (Andersen, personal communication) have provided strong evidence for a suppression of B. microti in short term in vitro cultures by cell populations harvested from the intraperitoneal cavity of mice harbouring patent S. mansoni infections in the presence of serum from B. microti infected mice, and this suggests that immunological factors may be involved in the suppression of B. microti by S. mansoni in vivo as shown in the present study. Other findings in line with this concept are the possible participation of non-specific immunological factors in the suppression of P. berghei in Trichinella spiralis infected mice (*Ngwenya* 1982) and the commonly demonstrated and by non-specific immunological factors mediated suppression of Plasmodium sp. and Babesia sp. by rickettsia, viruses and other pro-

tozoan species (see review by *Cox* 1975). The spleen is considered to play an important role in the regulation of Babesia sp. infections (*Schroeder et al.* 1966, *Todorovic et al.* 1967, *Phillips* 1969, *Roberts et al.* 1972) and since there is a marked splenomegaly in mice harbouring patent S. mansoni infections (*Mahmoud & Woodruff* 1972) being partly due to hyperplasia of the reticuloendothelial cells and lymphoid follicles (*WHO* 1974) it would be worthwhile to study the possible involvement of especially this organ in the suppression of B. microti in S. mansoni infected mice.

Experimental studies like the present using rodent models may provide valuable information about the presence and the nature of possible heterologous interactions of either antagonistic or synergistic nature arising as a consequence of concurrent infections with more parasite species. Such concurrent infections are widespread in domestic stock animals and man, and studies of the present type should be extended to domestic stock animals and appropriate primate models using relevant parasite combinations in order to elucidate the possible presence and consequences of such interactions in natural host-parasite relationships.

ACKNOWLEDGEMENTS

This study was supported by grants from the Carlsberg Foundation, from the Danish Natural Science Research Council and from the University of Ibadan, Ibadan, Nigeria. The technical assistance of Janne Vesterholt is greatly appreciated.

REFERENCES

Bailenger, J. & M. Guy: Interactions de deux parasitoses associées chez le rat: Plasmodium berghei et Strongyloides ratti. (Interactions between two parasite infections in the rat: Plasmodium berghei and Strongyloides ratti). Ann. parasitol. 1982, *57*, 513—516.

Bell, E. A.: A new method for counting Schistosoma mansoni eggs in faeces. Bull. WHO 1963, *29*, 525—530.

Buck, A. A., R. I. Anderson, A. A. MacRae & A. Fain: Epidemiology of poly-parasitism: I. Occurrence, frequency and distribution of multiple infections in rural communities in Chad, Peru, Afghanistan and Zaire. Tropenmed. Parasitol. 1978a, *29*, 61—70.

Buck, A. A., R. I. Anderson, A. A. MacRae & A. Fain: Epidemiology of poly-parasitism: II. Type of combinations, relative frequency and association of multiple infections. Tropenmed. Parasitol. 1978b, *29*, 137—144.

Cox, F. E. G.: Factors affecting infections of mammals with intraerythrocytic protozoa. Symp. Soc. exp. Biol. 1975, *29*, 429—451.

Fagbemi, B., N. Ø. Christensen & P. Nansen: Suppression of Babesia microti infection in mice concurrently infected with Fasciola hepatica. Vet. Parasitol. 1985, *17*, 101—110.

Hussein, H. S.: Babesia hylomysci in mice: Preference for erythrocytes of a particular age-group and pathogenesis of the anaemia. Z. Parasitenkd. 1976, *50*, 103—108.

King, M.: A Medical Laboratory for Developing Countries. Oxford University Press, London 1973.

Kloetzel, K., J. J. Faleiros, S. R. Mendes, C. T. Stanley & H. S. Arias: Concomitant infection of albino mice by Trypanosoma cruzi and Schistosoma mansoni. Parasitological parameters. Trans. Roy. Soc. trop. Med. Hyg. 1973, *67*, 652—658.

Landsberg, J. W. & L. C. Eskridge: The erythrocyte in Babesia canis. J. Parasitol. 1940, *26*, 377—385.

Lwin, M., C. Last, G. A. T. Targett & M. J. Doenhoff: Infection of mice concurrently with Schistosoma mansoni and rodent malarias: contrasting effects of patent S. mansoni infections on Plasmodium chabaudi, P. yoelii and P. berghei. Ann. trop. Med. Parasitol. 1982, *76*, 265—273.

Mahmoud, A. A. F. & A. W. Woodruff: Mechanisms involved in the anaemia of schistosomiasis. Trans. Roy. Soc. trop. Med. Hyg. 1972, *66*, 75—84.

McHardy, N.: Effects of stimulating erythropoesis in mice infected with Babesia rodhaini. Ann. trop. Med. Parasitol. 1973, *67*, 301—306.

Ngwenya, B. Z.: Enhanced resistance to Plasmodium berghei in mice previously infected with Trichinella spiralis. Parasite Immunol. 1982, *4*, 197—207.

Phillips, R. S.: The role of the spleen in relation to natural and acquired immunity to infections of Babesia rodhaini in the rat. Parasitology 1969, *59*, 637—648.

Roberts, J. A., J. D. Kerr & P. Tracey-Patte: Function of the spleen in controlling infections of Babesia rodhaini in mice. Int. J. Parasitol. 1972, *2*, 217—226.

Schroeder, W. F., H. W. Cox & M. Ristic: Anaemia, parasitaemia, erythrophagocytosis and haemaglutinins in Babesia rodhaini infection. Ann. trop. Med. Parasitol. 1966, *60*, 31—38.

Simons, H.: Sur les rapport des plasmodies paludiques et des piroplasmose avec les reticulocytes. (The relationship between plasmodia and piroplasms and the reticulocytes). Ann. Parasitol. 1939, *17*, 72—85.

Smithers, S. R. & R. J. Terry: The infection of laboratory hosts with cercariae of Schistosoma mansoni and the recovery of adult worms. Parasitology 1965, *55*, 695—700.

Todorovic, R., D. Ferris & M. Ristic: Roles of the spleen in acute plasmodial and babesial infections in rats. Exp. Parasitol. 1967, *21*, 354—372.

World Health Organization: Immunology of schistosomiasis. Bull WHO 1974, *51*, 553—595.

Willett, K. C.: An observation on the unexpected frequency of some multiple infections. Bull. WHO 1972, *47*, 747—749.

Yusuf, J. N., G. Pierkarski & B. Pelster: Concurrent infections of Trichinella spiralis and Toxoplasma gondii in mice. Z. Parasitenkd. 1980, *62*, 231—240.

SAMMENDRAG

Suppression af Babesia microti infektion hos mus med samtidig infektion med Schistosoma mansoni.

Babesia microti parasitæmien og haematologiske forandringer blev undersøgt hos henholdsvis mus med samtidig infektion med Schistosoma mansoni og hos kontrolmus uden schistosominfektion. B. microti parasitæmien blev suppreret i betydelig grad hos mus der var blevet inficerede med S. mansoni 6 og 8 uger tidligere. Denne suppression blev ledsaget af en reduktion/ophævelse af den af B. microti forårsagede reduktion i hæmoglobin og hæmatokrit niveauerne og af B. microti's bidrag til erythropoesens størrelse. Den suppressive effekt af patente S. mansoni infektioner på B. microti formodes i det mindste delvis at være forårsaget af non-specifikke immunologiske faktorer, men den af schistosominfektionen forårsagede ændring i erythrocytomsætningen sammenholdt med B. microti's præference for mature (gamle) erythrocytter kan muligvis også være en medvirkende årsag til suppressionen. I mus, der var blevet inficerede med S. mansoni 2 uger før infektionen med B. microti, forekom der ingen suppression af protozoinfektionen.

(*Received February 4, 1985*).

Reprints may be requested from: Niels Ørnbjerg Christensen, the Danish Bilharziasis Laboratory, Jægersborg Allé 1 D, DK-2920 Charlottenlund, Denmark.