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Thrombocytopenia: a uniform complication of African trypanosomiasis[1]

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Summary

Because an increasing number of reports have indicated that thrombocytopenia may occur during the course of African trypanosomiasis, a comprehensive study was designed to analyse the variables influencing the incidence and severity of this complication. Thrombocytopenia occurred in all of 40 infected domestic livestock and wildlife that were studied. The magnitude of the platelet reduction was independent of the route of inoculation (intravenous or transmission by tsetse), the genus or breed of livestock, and the species or variable antigen type (VAT) of trypanosome (several VATs of *Trypanosoma (Trypanozoon) rhodesiense*, *T. (T.) brucei*, *T. (Nannomonas) congolonse*, and *T. (Duttonella) vivax*. All 51 rats studied also became severely thrombocytopenic at the peak of parasitaemia with each of these 4 species and *T. (T.) gambiense*. The only variable that caused a statistically significant difference in the severity of thrombocytopenia was the height of parasitaemia, which was directly related to the reduction in the number of platelets. Rat platelets were more resistant than those of livestock to a given number of trypanosomes per ml of blood but became equally depressed during the course of the infection because the peak parasitaemia of rats was much higher. The data indicate that thrombocytopenia is a universal complication of African trypanosomiasis and underscore the potential importance of platelet damage in the pathogenesis of the coagulopathies, hemorrhage, vasoconstriction, and tissue damage that complicate this disease.

Key words: thrombocytopenia; African trypanosomiasis; platelet aggregation; disseminated intravascular coagulation (DIC).

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Table 1. Reports of thrombocytopenia in African trypanosomiasis

Trypanosome spp.	Mammal and (number)	Route of infection	DIC	Reference
T. rhodesiense	man (1)	tsetse	yes no	Barrett-Connor et al., 1973; Ottoman et al., 1970
	man (4)	tsetse	2 of 4	Robins-Browne et al., 1975
	rats, rabbits (many)	iv	no	Davis et al., 1974
	rhesus monkeys (9)	iv	N.S.	Sadun et al., 1973
T. gambiense	man (18)	tsetse	no	Greenwood and Whittle, 1976
	rats (10)	iv	N.S.	Greenwood and Whittle, 1976
T. brucei	rabbits (many)	iv	no	Jenkins et al., 1974
T. congolense	cattle (25)	iv	N.S.	Maxie et al., 1976
	cattle (20)	iv	yes	Wellde et al., 1978
	cattle (6)	iv	yes	Forsberg et al., 1979
T. vivax	cattle (25)	iv	N.S.	Maxie et al., 1976
	cattle (4)	iv	yes	Van den Ingh et al., 1976b
	goats (2)	iv	yes	Veenendaal et al., 1976

iv = intravenous; N.S. = not studied; DIC = disseminated intravascular coagulation

Introduction

Interest in thrombocytopenia and other life-threatening haemorrhagic complications of trypanosomiasis has increased since a young man who returned from East Africa to our hospital in San Diego, California, USA with Rhodesian trypanosomiasis almost died of disseminated intravascular coagulation (DIC) (Barrett-Connor et al., 1973). The platelets, fibrinogen and other coagulation factors of this patient were depleted so dramatically that he bled into his skin and other vital organs. When this strain of *Trypanosoma (Trypanozoon) rhodesiense* was inoculated into rats, it caused severe thrombocytopenia within 2–3 days without biochemical or clinical evidence of DIC (Davis et al., 1974). After the publication of these 2 studies, several subsequent reports have also suggested that thrombocytopenia either with or without DIC may be a frequent complication of African trypanosomiasis in human beings and animals (Table 1). Even without DIC, this thrombocytopenia may be severe enough to cause fatal hemorrhage (Robins-Browne et al., 1975).

Unfortunately, most of the reports listed in Table 1 did not establish that thrombocytopenia was a consistent feature of African trypanosomiasis because each studied only one species of trypanosome and one species of mammal. Furthermore, all the experimental infections were established by intravenous inoculation of bloodstream forms instead of infected tsetse flies and did not rule out the possibility that thrombocytopenia was associated primarly with large intravenous inocula of trypanosomes and their products. Accordingly, this

Table 2. Mammalian species and breeds tested against different species and variable antigen types of trypanosomes

Trypanosome species and variable antigen types	Number and breed of each species and animal			
	Rats	Cattle	Sheep/goats	Wildlife
T. rhodesiense				
Lump 139	10 Wistar			
ETat 1.10	10 Wistar	1 Friesian*	1 E. African goat	
ETat 1.3	10 Wistar			
T. brucei				
ILTat 1.4	10 Wistar	5 Friesian		1 Waterbuck 1 Buffalo
ILRAD 855			4 E. African goats*	
T. congolense				
Trans Mara I	10 Wistar			
ILRAD 958			6 Merino sheep 5 Red Masai sheep	
ILRAD 6-E8		6 Hereford		
ILRAD 588		2 Hereford		
T. vivax				
v 20	10 Wistar			
417			2 E. African goats*	
673		2 Boran 2 Hereford 2 Hereford*		
T. gambiense				
ILRAD 6	25 Wistar			

All rats were infected intraperitoneally. Cattle and goats denoted by an asterisk were infected by bites of infected tsetse (*Glossina morsitans morsitans*). All other animals were infected intravenously. The East African goats are Masai × Galla cross-breeds.

study was designed to determine if the incidence or severity of thrombocytopenia was influenced by the species or variable antigen type of trypanosome, the species of mammalian host, or the route of transmission.

Materials and Methods

Experimental animals: The species and breed of each animal used in the experiments are shown in Table 2. The male Wistar rats were raised in the ILRAD colony and weighed abbout 250 g at the time of infection. The Friesian, Hereford, and Boran cattle were all adults except for two 10-day-old Freisian calves that were challenged with *T. brucei* ILTat 1.4 and four 8-month-old Herefords that were challenged with *Trypanosoma (Duttonella) vivax* 673. The Masai-Galla crossbred goats, the Merino and Red Masai sheep, and the game animals were young adults.

Trypanosomes: Fourteen different strains and clones of *Trypanosoma* spp. were used (Table 2).

T. rhodesiense

T. rhodesiense ETat 1.3 and 1.10 are cloned derivatives of TREU 164; Lump 139 (ILRAD 853) was stabilated after 1 mouse passage from ETat 1.10 (Lumsden and Herbert, 1975).

T. (T.) brucei

T. brucei 1.4 is a monomorphic derivative of 227 stock (Barbet and McGuire, 1978). ILRAD 855 was cloned after several mouse passages from Lump 227 (ILRAD B-35, stabilated from UHEMBO/64/Eatro/795 stock).

T. (Nannomonas) congolense

Trans Mara I was isolated from an infected cow in the Trans Mara area near the Kenya-Tanzania border (Wellde et al., 1978). ILRAD 958 was derived after several mouse passages from STIB 212 which was isolated from a lion in the Serengeti area of Tanzania in 1971 (Geigy and Kauffman, 1973). ILRAD 6-E8 was stabilated after two mouse passages from EATRO 2226, isolated from a cow in Tanzania in 1974. ILRAD 588 was cryopreserved after one passage through irradiated mice of ILRAD 288, a cloned derivated of EATRO 209 stock (Morrison et al., 1982).

T. vivax

T. vivax 417 was derived from cyclical *G. morsitans* passage between goats of ILRAD V-17 (Emery and Moloo, 1981), a derivative of Zaria Y486 from Nigeria (Leeflang et al., 1976). ILRAD V-20 was stabilated after several mouse passages from V-17 and was in its 299th passage in rats. *T. vivax* 673 was isolated from a cow during an outbreak of haemorrhagic *T. vivax* infection in the coastal region of Kenya (Mwongela et al., 1981).

T. gambiense

T. gambiense ILRAD 6 was isolated from a human being in Zaire and passaged once through a rat before cryopreservation.

Infection: As shown in Table 2, cattle, sheep and goats were infected both by the intravenous route and by bites of infected tsetse (*Glossina morsitans morsitans*). Game animals were infected intravenously (iv) and rats intraperitoneally (ip). Rats were inoculated with 10^6 motile, cryopreserved trypanosomes except for *T. vivax* V-20 which was diluted to this concentration from the blood of an infected rat. Intravenous inoculations of cattle, sheep, goats, and wild game were usually with 10^5 or 10^6 motile, cryopreserved trypanosomes but the Herefords infected with *T. vivax* 673 (Table 2) were inoculated with 5×10^7 trypanosomes in 100 ml of blood from the 2 infected Borans (Table 2). Infections from tsetse were transmitted by bites from 5–10 tsetse that had previously fed on an infected goat.

Counting of platelets and trypanosomes: Platelets were counted by phase contrast microscopy in a hemocytometer after dilution of EDTA-treated whole blood (blood: 0.008 mg per ml EDTA = 9:1; Vol:Vol) with 1% oxalate and 0.1% brilliant cresyl blue. Trypanosomes from infected rats were counted by the same method. Parasitemia of infected livestock and wild game were estimated by the dark ground phase (DG) technique (Murray et al., 1977).

Experimental design: Infected rats were exsanguinated by cardiac puncture on the third day post-infection. Platelet and trypanosome counts were done on day 3, regardless of the degree of parasitaemia. The normal platelet count for the Wistar rats in the ILRAD colony was determined on blood obtained by cardiac puncture of 10 rats 3 days after ip inoculation of a 0.5 ml volume of rat blood diluted 1:5 with PSG. Ten uninfected rats were tested in the first week and again in the last week of the rat experiments. The two mean values did not differ significantly ($\pm$ 10%).

Domestic livestock and wildlife were bled by venipuncture into EDTA containing tubes 4–7 times per week for enumeration of platelets and trypanosomes. Normal platelet counts for cattle ($n = 15$), sheep ($n = 6$), and goats ($n = 7$) were established either from blood taken before infection or

Table 3. Thrombocytopenia in experimental trypanosomiasis

Trypanosome species	Mean of lowest platelet count $\times 10^3 \pm 1$ standard deviation			
	Rats	Cattle	Sheep/Goats	Buffalo/ Waterbuck
Uninfected	899 ± 126 (20)	541 ± 100 (15)	467 ± 42 (6)/427 ± 72 (7)	704/469
range	702 – 1128	404 – 823	416 – 523/354 – 553	–
T. rhodesiense	146 ± 80 (20)	130 (1)	164 (1 goat)	–
range	78 – 348	–	–	
% reduction	84	75	65	
p value	<0.001	–	–	
T. brucei	196 ± 97 (9)	116 ± 91 (5)	135 ± 84 (4 goats)	148/223 (1 each)
range	54 – 380	65 – 278	29 – 244	
% reduction	78	79	69	69/69
p value	<0.01	<0.01	<0.05	–
T. congolense	125 ± 54 (5)	120 ± 33 (8)	148 ± 52 (11 sheep)	–
range	55 – 178	76 – 182	90 – 235	
% reduction	86	78	70	
p value	<0.001	<0.01	<0.05	
T. vivax	86 ± 57 (8)	27 ± 15 (6)	78 ± 23 (2 goats)	–
range	50 – 212	7 – 42	61 and 94	
% reduction	90	96	82	
p value	<0.001	<0.001	–	
T. gambiense	291 ± 89 (9)	–	–	–
range	138 – 467			
% reduction	68			
p value	<0.02			

All infected domestic livestock and wildlife are included. The numbers in parentheses are the total number of animals in each group. Only rats that had reached a peak parasitaemia of 10^5 per mm^3 of peripheral blood are shown. The figures given for rats infected with *T. rhodesiense* are the mean for all 3 VATs shown in Table 2. The strain and VAT used to infect each species of animal is shown in Table 2. p values were determined from Student's t-test.

concurrent normal controls. The pre-inoculation samples from the 2 ten-day-old Friesian calves were not included. The pre-infection count of the buffalo and water buck were taken as normal for these species.

Statistical analysis of data: Student's t-test was used for all statistical calculations.

Results

All infected animals became thrombocytopenic. The mean platelet counts, ranges, and percent reductions from normal controls for each group of animals infected with each species of trypanosome are shown in Table 3.

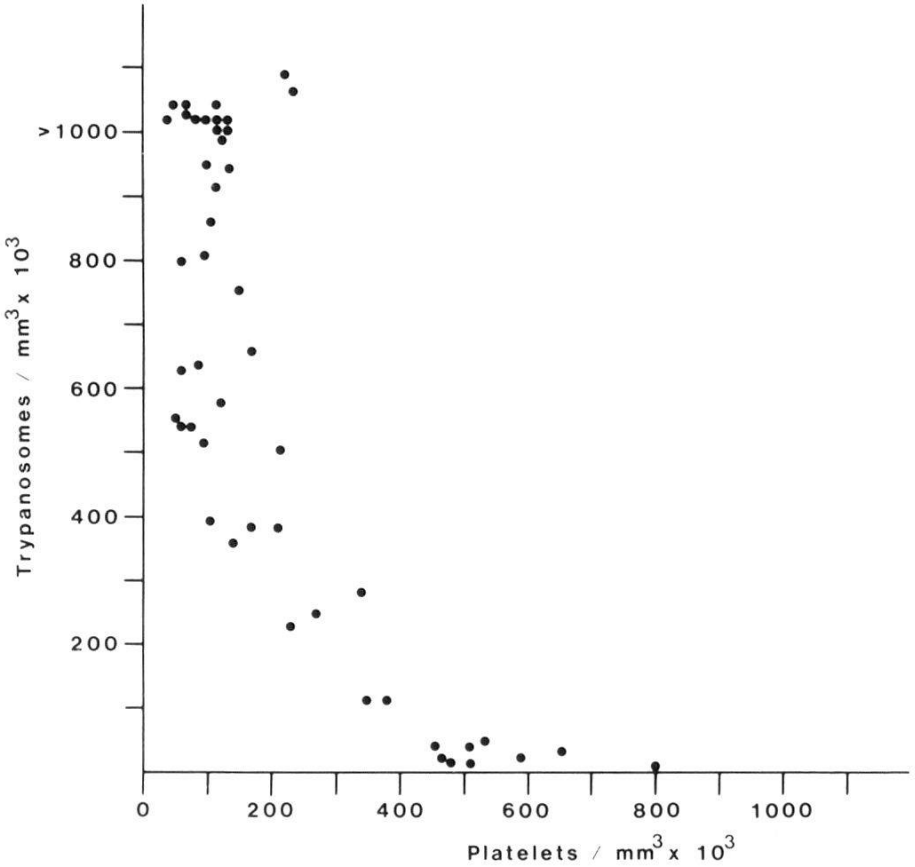

Fig. 1. Dose response relationship of rat platelets to numbers of trypanosomes per ml of blood. Each point represents the platelet count of one rat at the designated level of parasitaemia, regardless of the species of trypanosome.

Rats: Rats were severely thrombocytopenic at the first peak of parasitaemia. Since all rats were sacrificed 3 days after infection, however, some had not reached a peak of parasitaemia. The values for rats included in Table 3 are those for which parasitaemia was recorded as greater than 10^8 trypanosomes per ml of blood. There was no statistical difference in the degree of thrombocytopenia of rats infected with any species or VAT of trypanosome. The mean platelet counts in Table 3 for rats infected with *T. rhodesiense* were derived from *T. rhodesiense* LUMP 139 ($167 \pm 94 \times 10^3$ per mm^3), ETat 1.10 (112 ± 41) and ETat 1.3 (159 ± 106).

The effect of the level of parasitaemia on the platelet counts of rats is shown in Fig. 1, in which the platelet counts of all infected rats are plotted against parasitaemia per ml of blood, regardless of the species or VAT of trypanosome. The severity of thrombocytopenia was directly related to the height of parasitaemia. The uniformity of the points in Fig. 1 also emphasises that each species and VAT of trypanosomes caused similar degrees of thrombocytopenia at equivalent levels of parasitaemia.

Domestic livestock and wildlife: The values given for infected domestic livestock and wildlife in Table 3 were calculated from the lowest platelet count of each individual during the course of infection. These animals were always

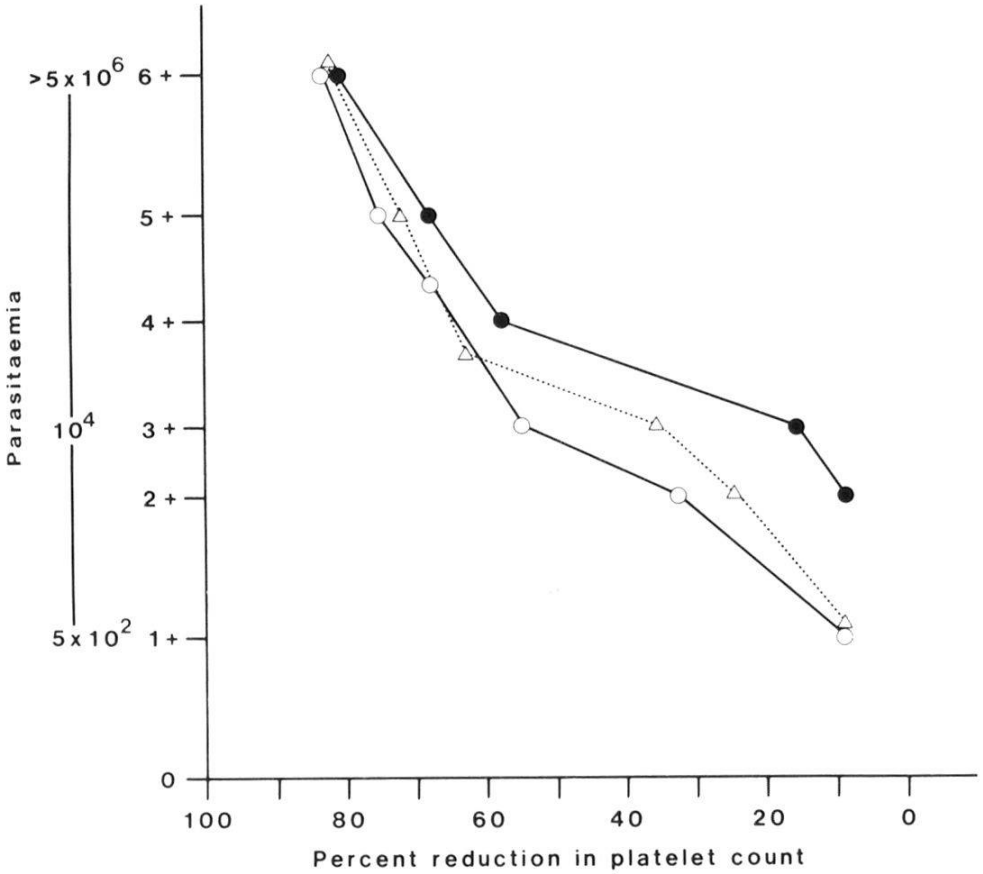

Fig. 2. Dose response relationship: Percent reduction of platelet counts to numbers of trypanosomes per ml of blood in domestic livestock. The mean percent reduction in serial platelet counts of all domestic livestock is plotted against the parasitaemia at the time these platelet counts were done. Thus, each point represents the mean of all platelet counts at the designated level of parasitaemia, regardless of the species of trypanosome. Cattle = ●; sheep and goats = ○; mean of both = △.

severely thrombocytopenic at the first peak of parasitaemia and most of the platelet counts included in Table 3 were taken at that time.

Analysis of the figures in Table 3 shows that all parasitaemic, infected domestic animals and wildlife become severely thrombocytopenic regardless of the route or mode of inoculation (tsetse vs. iv), the species of animal, and the species or VAT of trypanosome. The percent reduction in the platelet counts of animals infected by tsetse was exactly the same as that of animals infected by the iv route (77%). The slight difference in the reduction of the platelets of sheep and cattle was not statistically significant. *T. rhodesiense*, *T. brucei*, and *T. congolense* caused comparable degrees of thrombocytopenia (69–78%) in livestock. Although there tended to be a greater reduction in the platelet counts of animals infected with *T. vivax*, this difference was not statistically significant. It is interesting, however, that the East African strain of *T. vivax* used to infect the cattle in this experiment causes haemorrhagic disease of cattle in the coastal region of Kenya (Hudson, 1944; Mwongela et al., 1981).

On the other hand, the number of trypanosomes per ml of blood profoundly influenced the severity of thrombocytopenia (Fig. 2). The values given in this

figure were obtained from serial platelet and trypanosome counts on all infected animals, regardless of the species or VAT of trypanosome. Only counts obtained up to the first peak of parasitaemia were included because severe disease with frequent waves of parasitaemia prevents the platelet count from returning to normal even when the animal is aparasitaemic and animals that undergo selfcure sometimes develop a transient increase in platelets (data not shown). The reductions in the mean platelet counts were directly related to the degree of parasitaemia. Each increase in parasitaemia of greater than 1 $\log_{10}$ per ml peripheral blood caused a significant reduction in the platelet count (p values from <0.05 to <0.001). The platelets of sheep and goats are as susceptible to moderate and high parasitaemias as those of cattle. Goats and sheep developed slightly less severe thrombocytopenia at low levels of parasitaemia but the differences are fairly small and the mean reductions in platelet counts were established on fewer samples than those with moderate and severe thrombocytopenia.

Discussion

This study has shown that thrombocytopenia is a universal complication of experimental African trypanosomiasis. It occurs during parasitaemia, regardless of the species of trypanosome, the route of inoculation or the species or breed of infected mammal (Table 3 and Fig. 2). The severity of thrombocytopenia in domestic livestock and rats during the early phase of infections with *T. rhodesiense*, *T. brucei*, *T. vivax*, *T. congolense*, and *T. gambiense* is influenced dramatically only by the degree of parasitaemia (Fig. 1 and 2). The platelet count falls as the parasitaemia rises.

Although the platelets of rats are profoundly more resistant than domestic livestock to equivalent levels of parasitaemia, severe thrombocytopenia is a feature of trypanosomiasis in all livestock with a parasitaemia of greater than 10^5 trypanosomes per ml of blood and all rats with a parasitaemia of greater than 10^8 per ml. Furthermore, the dose response curve of rat platelets to the number of parasites per ml of blood parallels that of livestock after the essential threshold number of parasites is reached (compare Fig. 1 and 2). It is also interesting that this threshold is approached near the usual peak of parasitaemia for both rats (10^8 to 10^9 per ml) and domestic livestock (5×10^4 to 10^6). In any event, thrombocytopenia is almost certainly also a universal complication of clinical trypanosomiasis in the field since the depressions of the platelet counts in this study were just as severe when the disease was transmitted by tsetse and occurred at realistic levels of parasitemia (Fig. 2).

Thrombocytopenia is probably caused by aggregation of platelets. Davis et al. (1974) showed by phase and electron microscopy that whole trypanosomes and trypanosome-free sonicates of *T. rhodesiense* caused marked platelet aggregation in vitro, independently of ADP, complement, kinins, antibody, fatty

acids, and the presence of the spleen. The present study has strengthened the hypothesis that platelet aggregation plays an important role in the thrombocytopenia of trypanosomiasis because there was significant aggregation of platelets in the haemocytometer counts from all 40 of the domestic livestock and wildlife (data not shown), regardless of the speed with which the samples were processed. Aggregation of platelets from naive animals by trypanosome-free supernatants of disrupted *T. rhodesiense*, the appearance of thrombocytopenia in rats before antibody formation (Davis et al., 1974), and a shortened life span of bovine platelets in cattle infected with *T. congolense* (Preston et al., 1982) all suggest that the thrombocytopenia is caused by trypanosomal products or components. Platelets coated with trypanosomal antibody will also aggregate in the presence of the specific trypanosomes (Rickenberg, 1917), and immune complexes of *T. vivax* antigen and antibody will induce aggregation and release of radiolabeled serotonin from platelets (Slots et al., 1977). These observations suggest that there may be more than one mechanism of platelet aggregation during the course of trypanosomiasis.

The relationship of thrombocytopenia to DIC is less clear. Several studies have now shown either biochemical or pathological evidence of intravascular coagulation during infection of human beings with *T. rhodesiense* (Barrett-Connor et al., 1973; Robins-Browne et al., 1975) and experimental animals with *T. congolense*, *T. brucei*, *T. vivax* and *T. simiae* (Wellde et al., 1978; Boreham and Facer, 1974; Forsberg et al., 1979; Veenendaal et al., 1976; Van den Ingh et al., 1976a, 1976b; Isoun, 1975). Some workers have suggested that these abnormalities of the fluid phase coagulation factors may be due to autoantibodies against fibrinogen (Rickman and Cox, 1979; Thoongsuwan et al., 1979) or the action of immune complexes on the contact factors of the first phase of the coagulation cascade (Boreham and Facer, 1974). While these events undoubtedly occur, thrombocytopenia precedes the other coagulation abnormalities in livestock (Wellde et al., 1978; Forsberg et al., 1979) and occurs by the second and third days after infection in rats before antibody could be produced (Davis et al., 1974; this study). Since platelets release a phospholipid, platelet factor 3, that triggers the coagulation cascade, it seems likely that the coagulation disorder of trypanosomiasis has begun before the production of autoantibodies or immune complexes.

Now that it has been established that thrombocytopenia is a universal complication of trypanosomiasis during parasitaemia, future studies should concentrate on the mechanisms of thrombocytopenia, the role of platelets in the haemorrhagic syndrome of trypanosomiasis in human beings (Barrett-Connor et al., 1973; Robins-Browne et al., 1975) and cattle (Hudson, 1944; Mwongela, 1981), activities of platelets that may influence the height of parasitaemia and other possible roles of platelet destruction in the pathogenesis of trypanosomiasis. For example, platelet aggregation releases serotonin which is a powerful vasoconstrictor. Veenendaal et al. (1976) have shown that platelet aggregation

and fluctuations of the blood serotonin levels occurred during temperature spikes associated with peaks of parasitaemia in goats infected with *T. vivax*. Goodwin and Hook (1968) showed that the small arteries of rabbits infected with *T. brucei* were constricted and postulated that this vasoconstriction was responsible for the extensive tissue damage associated with *T. brucei* infections. Although they hypothesized that histamine and catecholamines might be responsible for this vascular damage, subsequent studies of thrombocytopenia and platelet aggregation strongly suggest that serotonin may be an important mediator. Thus, platelet aggregation and thrombocytopenia may contribute to many aspects of the pathogenesis of trypanosomiasis, in addition to playing a critical role in the induction of the complications of DIC and haemorrhage.

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