# Some effects of uninfected laboratory-reared tsetses ("Glossina morsitans morsitans" Westw.) (Diptera: Glossinidae) on host-rabbits

Autor(en): **Parker, K.R.**

Objekttyp: **Article**

Zeitschrift: **Acta Tropica**

Band (Jahr): **35 (1978)**

Heft 4

Acta Tropica 35, 345–355 (1978)

Department of Entomology, University of Alberta, Edmonton, Alberta, Canada T6G 2E3

# Some effects of uninfected laboratory-reared tsetses (*Glossina morsitans morsitans* Westw.) (Diptera: Glossinidae) on host-rabbits

K. R. PARKER

## Summary

Rabbits (Flemish giant x French lop-eared) exposed to 300 to 500 tsetses (*Glossina morsitans morsitans* Westw.) a day, 2 or 3 days a week, did not show significant differences from littermates receiving no exposure, with respect to weight changes, haematocrits, red and white blood cell counts or whole blood clotting times. In other rabbits, the same daily exposure, 6 days a week, resulted in sharp decreases in haematocrit levels and in some, changes in weights, but no change in citrated plasma thrombin times. Rabbit haematocrit levels correlated negatively with estimated weekly blood loss. Weights and haematocrits of most rabbits exposed to 1,200 to 1,500 flies on a single occasion were not affected. However, following heavy exposure, ears were cold and blood letting difficult, indicating the possibility of arteriolar vasoconstriction.

*Key words: Glossina;* tsetse; tsetse husbandry; host-response; biting fly; haematocrits; weight changes; clotting times.

## Introduction

Although most tsetse colonies are presently maintained using in vivo feeding techniques, there are apparently no publications on the physiological responses of the hosts. Itard and Jordan (1977) stated that host-rabbits, used to feed 1,000 to 1,200 tsetses one day a week at the Maisons-Alfort Laboratory (France), do not lose weight and maintain an adequate longevity.

Because of the paucity of information in this area, this research was designed to examine some of the physiological responses of rabbits exposed to tsetses, specifically weight changes, haematocrit levels, and plasma clotting

Correspondence: K. R. Parker, Department of Entomology, University of Alberta, Edmonton, Alberta, Canada T6G 2E3

times. Physiological abnormalities in hosts, caused by excessive tsetse exposure, can affect tsetses subsequently feeding on these hosts (Parker, 1978). Furthermore, results of laboratory-controlled biting-fly exposure of this type may be applied to host/biting-fly interactions occurring under natural conditions. Adequate information on host/biting-fly relationships is essential, but unfortunately not available, for determining which levels of arthropod infestation require application of control measures (Steelman, 1976).

**Materials and methods**

The colony of uninfected *Glossina morsitans morsitans* Westw. originated from pupae obtained from the Tsetse Research Laboratory, University of Bristol, England, in 1973. Since then, a closed colony of 1,000 to 2,000 flies, mostly females, has been maintained in the Department of Entomology, University of Alberta, on a strain of rabbits originated from a cross of Flemish giant x French lop-eared rabbits. Maintenance procedures were those described by Nash et al. (1971). Flies (15/cage) were placed on the ears or clipped backs of rabbits for 15–20 min each day, 6 days a week. Rabbits were used from age 6 months and exposed, on average, 2–3 days a week and at most 6 days a week, for up to 5 h per day. Rabbits were maintained at $17 \pm 1.3°$ C on a 15–9 h day-night cycle and fed a standard lab diet of Master Baby rabbit pellets (18% protein; Maple Leaf Mills, Edmonton) containing no antibiotics or coccidiostats. Food and water were available ad libitum.

Rabbit weights ($\pm 2.5$ g) were obtained using a full capacity beam scale (Morris Scale Co., Portland, Oregon). Food and water were not restricted prior to weighings. Blood samples were obtained from the marginal artery of the left ear. Duplicate heparinized haematocrit capillary tubes (Pre Cal, Dade Division, Fla.) were filled directly from the puncture wound following withdrawal of the needle and centrifuged at 3700 *g* for 4 min. Red and white blood cell counts (Benson and Gunstream, 1970) were obtained with a Levy corpuscle counting chamber and Neubauer grid (C. A. Hausson and Son, Pa.). Thrombin times (Fletcher et al., 1959) were performed at 37° C using 3 NIH unit/ml bovine thrombin (Parke-Davis, Brockville, Ontario) in saline and citrated rabbit plasma. Capillary (Benson and Gunstream, 1970) and tube (Lee and White, 1913) whole blood clotting times were performed at $17 \pm 1.3°$ C.

*Experimental design*

*Rabbits exposed to tsetses 2 to 3 times a week.* Twelve male rabbits from 3 litters were used. Equal numbers of control (no exposure) and experimental (exposed) rabbits were obtained from each litter. Experimental rabbits were exposed to 300 to 500 tsetses per day, 2 or 3 days a week, for a period of 8 months. Physiological parameters of all rabbits were determined at intervals over the 8 months.

*Rabbits exposed to tsetses 6 days a week.* Two male rabbits from separate litters, and one male and one female from a third, were exposed to 250 to 500 tsetses daily, 6 days a week, for 20 weeks. Three control rabbits, one male from each of the litters from which experimental rabbits were obtained, were held without exposure to tsetses for 11 weeks. During the tenth week, a control male ($C_1$) died for no apparent reason (no necropsy performed), and was replaced by a previously unexposed female littermate ($C_{1'}$). During the twelfth to fifteenth week, the previously unexposed control rabbits were exposed to 120 flies per day, for 6 days, to determine the effects of short, low levels of exposure. Control and experimental rabbits were bled (10 ml) once a week, on the only day experimental rabbits received no exposure. Control rabbits were bled from weeks 5 to 20 only. Records of rabbit weight and haematocrit were collected weekly. Citrated plasma was used for determining thrombin times. The number of flies fed on each rabbit was recorded daily and used for estimates of host-blood loss.

*Rabbits exposed to 1,200 to 1,500 flies in 4 h.* Ten rabbits, from 1 to 3 years of age were used.

Three rabbits were exposed for the first time, while 3 had received fly exposure prior to this experiment. The remaining 4 rabbits, all of which had received no previous exposure, were used as controls, and were handled in the same manner as exposed rabbits, except that cages strapped to their backs and ears contained no flies. Flies were starved 48 h prior to feeding. One rabbit was exposed each day. Four cages of flies, placed on rabbits' ears and backs, were changed every 5 min for approximately 4 h. The number of flies which fed was estimated visually. Although 1,200 to 1,500 flies were applied, I estimated that only 900 to 1,125 fed. Rabbit weight, haematocrit, and a blood sample were taken from all rabbits immediately pre- and post-exposure (or control handling) and periodically for 18 to 30 days post-exposure.

## Results

*Rabbits exposed 2 to 3 days a week for 8 months*

The difference between control (non-exposed) and experimental (exposed) rabbits was not significant (Student's t; $p \geqslant 0.05$) with respect to red and white cell counts, haematocrits, weight changes, and whole blood clotting times (Table 1). Mean weight gains for control and experimental rabbits were +30.2 and +4 g respectively, however, one exposed rabbit lost 18% (720 g) of its initial body weight over the 8 month period. Without this instance of weight loss, experimental rabbits would have gained more than the controls. Values of all parameters were within those reported by previous authors (see review by Scarborough, 1931).

*Rabbits exposed 6 days a week for 20 weeks*

Over a 20 week period, each of the 4 rabbits (exposed to 250–500 flies per day, 6 days a week) were subjected to the equivalent of approximately 40,000 tsetses, of which an estimated 75% fed. During this experiment, weight of one male did not change; one male lost 330 g during the first 2 weeks, regaining half this loss in the following 6 weeks; and a third male lost 10% (534 g) of its weight by week 12 then kept its weight constant. The female gained weight reaching peaks during the 7th and 14th weeks of exposure. Maximum weight gain (478 g) of 8.3% initial body weight occurred during the 14th week. Weights of the 3 control rabbits remained relatively constant except during the week each was exposed to tsetses. At this time, control rabbits lost 50 to 100 g, equivalent to 2 to 5 times the estimated weight of blood taken by the tsetses.

Haematocrits of all 4 heavily exposed rabbits decreased sharply and for the most part remained well below haematocrits of sibling controls (Fig. 1). Haematocrits of the 3 control rabbits remained, on average, above 40% despite weekly bleeding, except for a period between the 12th and 15th weeks, when each was exposed for 6 days (Fig. 1). During this period, haematocrits dropped 2 to 4%. Based on daily tsetse exposure, an estimate that 75% of the flies fed daily, and an average meal weight for *G. morsitans* of 0.034 g (Lester and Lloyd, 1928), a weekly estimate of the blood loss due to tsetses was obtained. During the 20 weeks, each heavily exposed rabbit lost an estimated 1000 g of blood (excluding

Table 1. Physiological parameters of control rabbits and rabbits exposed to tsetses 2 to 3 times a week over a period of 8 months

| Parameter | Time after first exposure | Control rabbits (no exposure) | | Experimental rabbits (exposed) | | Student's t |
|---|---|---|---|---|---|---|
| | | $\bar{x}$* | S.D. | $\bar{x}$* | S.D. | |
| Weight (kg) | 5 wk | 4.77 | 0.29 | 4.59 | 0.15 | 1.20 |
| | 32 wk | 4.86 | 0.30 | 4.59 | 0.33 | 1.24 |
| Red cells ($10^6/\mu l$) | 6 wk | 8.70 | 0.97 | 7.65 | 1.09 | 1.61 |
| White cells ($10^3/\mu l$) | 9 wk | 8.69 | 1.32 | 8.06 | 0.57 | 0.99 |
| Haematocrit (%) | 5 wk | 39 | 1.4 | 38 | 2.2 | 0.91 |
| Coagulation time: | | | | | | |
| Capillary (sec) | 6 wk | 270 | 31 | 216 | 48 | 2.10 |
| | 9 wk | 273 | 20 | 243 | 41 | 1.45 |
| Tube (min) | 18 wk | 16.25 | 3.17 | 16.33 | 4.70 | 0.03 |

* All means are averages of 6 values. All rabbits are males.

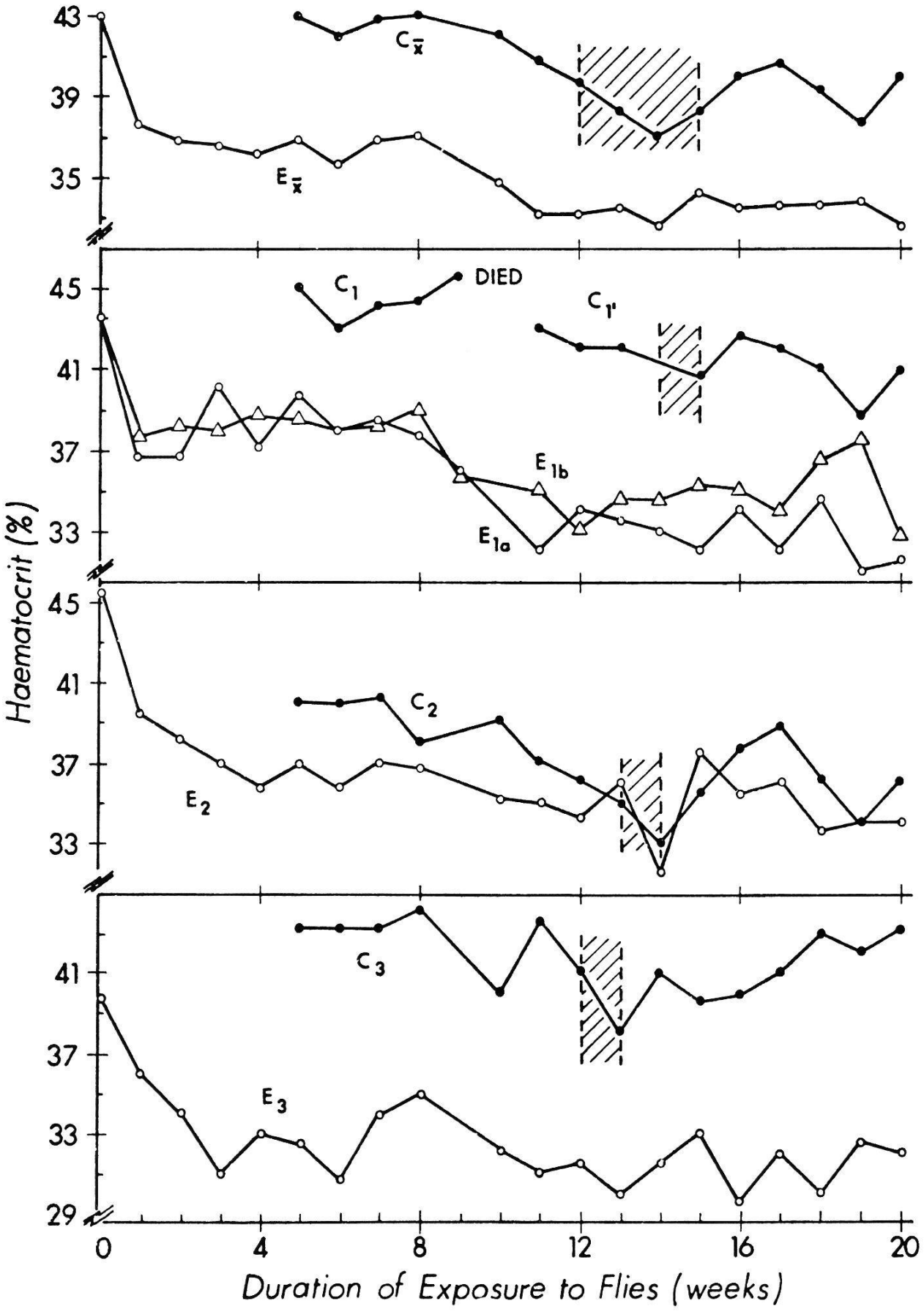


Fig. 1. Haematocrits of control (C) and experimental (E) rabbits. Experimental rabbits were exposed to 300–500 tsetses 6 days a week while control rabbits received only one 6 day exposure each, of 120 flies per day (crossed-hatched area). Top figure indicates mean values. Arabic numbers represent litters. Rabbits are grouped with sibling controls.

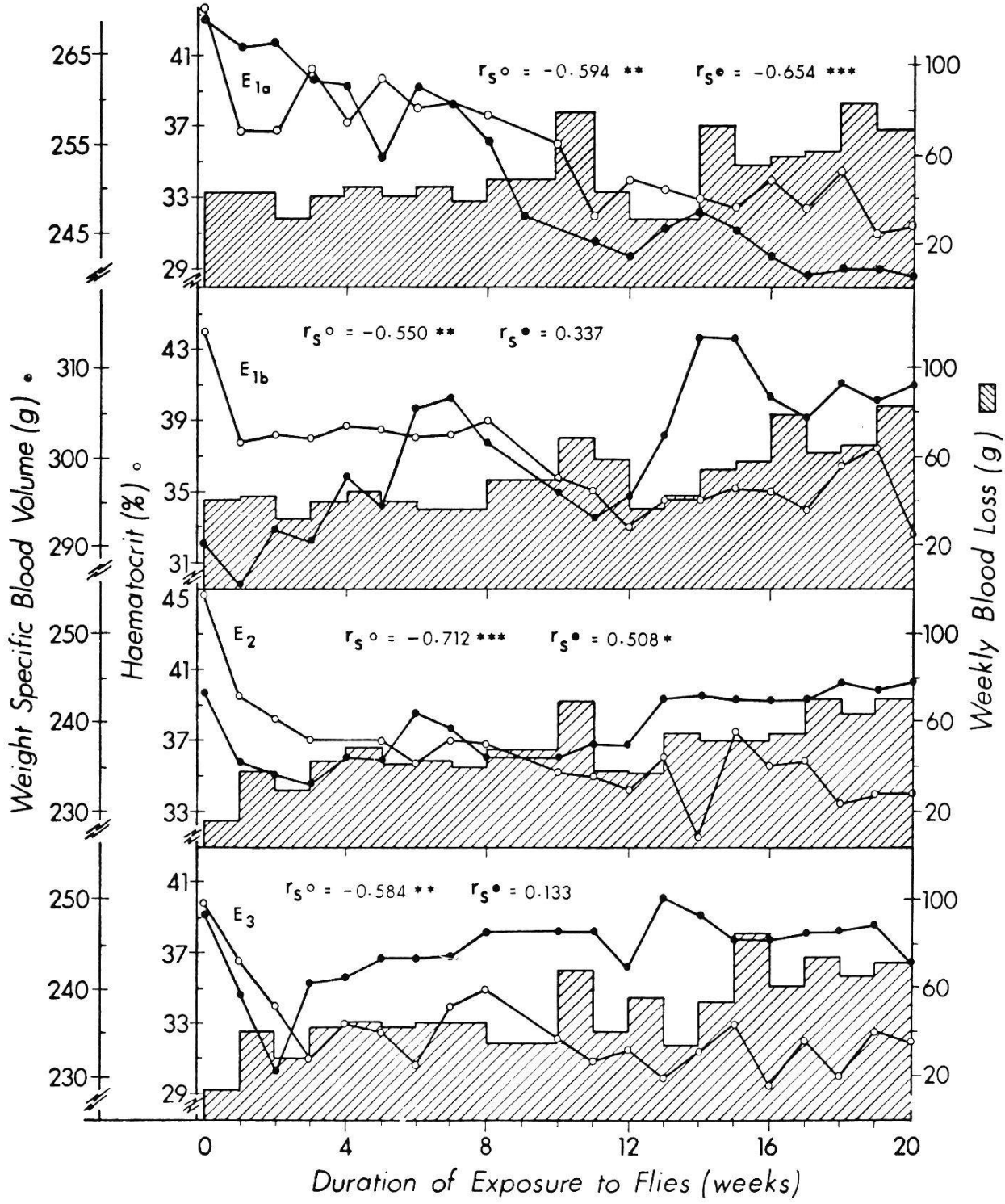


Fig. 2. Correlation of haematocrits and weight specific blood weight with estimated blood loss due to tsetses in rabbits exposed to 300 to 500 tsetses a day, 6 days a week. Spearman's correlation coefficients ($r_s$) were calculated using haematocrit and weight specific blood weights at the end of each week, and weekly estimated blood loss. Level of significance $p \leq$ *0.05; **0.01; ***0.001.

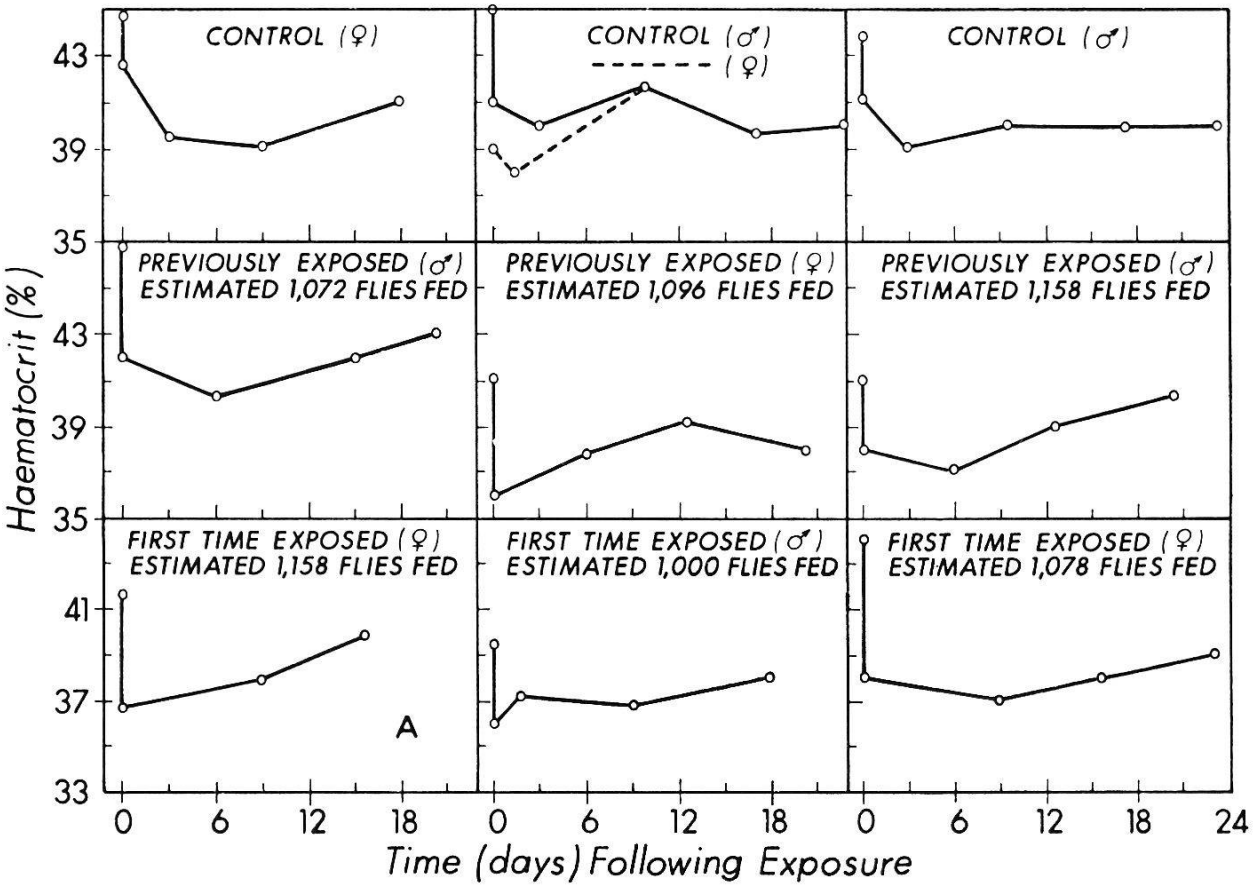


Fig. 3. Haematocrits of rabbits exposed to 1,200 to 1,500 flies within a 4 h period. On day 0, rabbits were bled pre- and post-exposure.

the 10 ml removed each week for serum and plasma samples). This loss is roughly equivalent to 4 times the rabbit's total blood weight (at any one time) estimated from the weight specific formula 0.055 $B^{0.99}$ (Spector, 1956). The 10 ml of blood removed at weekly bleeding represents the weight of blood in another 175 tsetse blood meals per week. Although correlation existed between weekly blood loss due to tsetses and weekly weightspecific blood weight in 2 of the 4 rabbits (Fig. 2), it was not consistently concordant or discordant. A significant negative correlation was obtained between weekly blood loss due to tsetses and weekly haematocrit values for heavily exposed rabbits (Fig. 2).

Thrombin times of citrated plasma, taken at weekly intervals from heavily exposed rabbits, fluctuated between 11 and 18 sec while those of the 3 control rabbits fluctuated between 11 and 19 sec. There was no tendency for the thrombin times to change in response to initial or varied exposure.

*Rabbits exposed to 1,200 to 1,500 tsetses in 4 h*

Weights of exposed and control rabbits on the day of exposure, or control handling, and for a period of 18 to 30 days post-exposure, responded similarly, with the exception of one, first time exposed, female rabbit. This rabbit experienced an 11% (730 g) increase in weight by the 18th day following exposure.

A female littermate receiving only control handling had a 6% (335 g) weight increase by the 24th day post-control handling. The other 8 rabbits showed either no weight change or increased weight slightly during the 3 weeks post-exposure.

Haematocrits of all 10 rabbits responded similarly (Fig. 3). Control rabbit haematocrits declined 2 to 4% as a result of pre- and post-control handling and bleeding. In the exposed rabbits, receiving exposure to 1,200 to 1,500 flies in addition to bleedings, haematocrits dropped 3 to 6%. Rabbits heavily exposed for the first time, responded similarly to rabbits with previous exposure. During the 3 to 4 weeks post-exposure, haematocrits of one control, one previously-exposed rabbit, and 2 rabbits exposed for the first time, returned to pre-exposure levels. The same rabbit which demonstrated the sharp increase in weight also showed the sharpest decrease in haematocrit (Fig. 3A). Haematocrit values of this rabbit returned to normal by the 16th day post-exposure.

Attempts were made to obtain blood for preparing citrated plasma immediately post-exposure. Ears were cold and bleeding from ears difficult, resulting in poor bleedings and clotting of citrated blood. Xylene, placed on the tip of the ear (and later removed) failed to cause dilation of the blood vessels.

## Discussion

Weights and haematological parameters were not significantly affected in rabbits exposed to 300 to 500 tsetses, 2 or 3 days a week. The same exposure, 6 days a week, resulted in decreases in haematocrits and, to a lesser extent, changes in weight. The high level of exposure required to induce host response and the failure of host parameters to change dramatically may be, in part, a result of the size of the rabbits (4.7–6.2 kg) and the high protein (18%) diet on which they were maintained. Hosts receiving deficient diets have reductions in growth, antibody production, haematopoiesis, and circulating globulins, subsequently leading to a breakdown of resistance (Nelson et al., 1977). Since haematological responses did not reach detrimental levels, and since antibody response in these rabbits develops quickly (Parker, 1978), host nutritional intake was probably more than sufficient to maintain metabolic processes, even during heavy tsetse exposure.

Seebeck et al. (1971) performed experiments with cattle to separate the effects of reduced food intake (anorectic effect) from those due to the remaining factors (specific effects) of tick *(Boophilus microplus)* infestation. The anorectic effect accounted for approximately two thirds of the weight losses due to tick infestation. In the present study, weights of 2 female rabbits increased, perhaps due to a specific factor of tsetse exposure affecting female, but not male host metabolism (possibly osmoregulation and water retention). Further studies will be required to substantiate this possibility.

Three control rabbits, receiving only one week of tsetse exposure, lost 2 to 5

times the estimated weight attributed to the blood meals taken by the tsetse during the one week of exposure (estimated on the assumption that all flies fed and that rabbits did not compensate for this by increased food consumption). Since control rabbits received low levels of exposure to tsetses for only one week, this additional weight loss is more likely to be the result of an anorectic rather than a specific response. Prolonged exposure (as with the 4 heavily exposed rabbits) would be more likely to affect host metabolism, possibly via salivary toxins.

Haematocrits of cattle progressively decreased under light moderate, and heavy exposures to the louse, *Haematopinus eurysternus* (Collins and Dewhirst, 1965). Similarly, rabbit red cell volume decreased with increased tick exposure (Jellison and Kohls, 1937). Apparently no similar studies with biting-flies have been reported. In the present study, haematocrit levels of rabbits exposed 6 days a week for 20 weeks, correlated discordantly with weekly estimated blood loss due to tsetses (Fig. 2).

Nelson and Haufe (unpubl., from Nelson et al., 1977) have noted that anemia in cattle is not always correlated with louse numbers. Nelson et al. (1977) suggest that this may indicate that a toxin is involved in the development and maintenance of louse-induced anemia in cattle. The present study demonstrated a correlation between rabbit haematocrits and tsetse exposure, suggesting that anemia is due to blood loss, although salivary toxins might also be affecting red cell production.

Van Handel (1962) suggested that perhaps the intermittent feeding of blood-sucking insects, and consequently the injection of their anticoagulants into man, may contribute to a decrease in coronary thrombosis. With this in mind, blood clotting times were examined to determine if the potent tsetse anticoagulant (Parker, 1978) prolongs coagulation. Whole blood clotting times of rabbits exposed to tsetses 2 to 3 days a week were not significantly different from non-exposed controls. Similarly, plasma thrombin times of heavily exposed rabbits were not consistently or significantly different from control rabbits. Nelson et al. (1970) reported that whole blood of cattle made anemic by *Haematopinus eurysternus*, clotted more rapidly than whole blood of cattle which were louse-resistant (harbouring low louse infestations). The possibility that the tsetse anticoagulant (Parker, 1978) affects the in vivo clotting times of rabbits seems unlikely for 3 reasons. First, the tsetse is a telmophage (Gordon and Crewe, 1948), probably feeding from a pool of blood rather than directly from a blood vessel, thus little of the anticoagulant probably reaches the vascular system at one time. Second, the tsetse sucks back much of the injected saliva with the blood meal (Lester and Lloyd, 1928), reducing the probability that a significant amount remains in the host. Third, foreign substances such as the tsetse anticoagulant, which do reach the vascular system, would probably be excreted by the kidneys, as hirudin is, within a short time following intravenous injection (Markwardt from Seegers, 1967).

Although close examination of host cutaneous responses was not part of this study, a few observations were made. Except for one rabbit, bleeding of previously-exposed rabbits was no more difficult than bleeding rabbits with no previous exposure. Following exposure, the ears of rabbits exposed to tsetses did not show signs of irritation or abnormality. No dry, scaly, or oedematous conditions, similar to those described in goats exposed to tsetses (Nash et al., 1965) were observed. Only one rabbit was difficult to bleed. This rabbit's ears became thick skinned and hard, and bleeding from the ear was difficult, even with the use of xylene. In all 6 rabbits exposed to 1,200 to 1,500 tsetses in a 4 h period, bleeding post-exposure was very difficult. Xylene failed to remedy the problem. Following withdrawal of the needle, bleeding seldom occurred. Citrated blood that was collected often clotted, even after periods of prolonged mixing, probably as a result of unclean venipuncture. It is probable that these results indicate the development of arteriolar vasoconstriction, although further experiments will be required to substantiate this possibility.

*Acknowledgments.* I thank R. H. Gooding and B. S. Heming, Department of Entomology, University of Alberta, and W. A. Nelson, Agriculture Canada, Research Station, Lethbridge, Alberta, for constructive criticism of this manuscript. I thank Miss M. I. Buick and Mrs. B. Burridge for maintenance of the tsetse colony. This research was supported, in part, by grants from the National Research Council of Canada (No. A-3900) and from Agriculture Canada (No. 6010) awarded to Dr. R. H. Gooding.

1 Benson H. J., Gunstream S. E.: Anatomy and physiology laboratory textbook. Wm. C. Brown Publishing Co., Iowa 1970.
2 Collins R. C., Dewhirst L. W.: Some effects of the sucking louse, *Haematopinus eurysternus*, on cattle on unsupplemented range. J. Amer. vet. med. Ass. *146*, 129–132 (1965).
3 Fletcher A. P., Alkjaersig N., Sherry S.: The maintenance of a sustained thrombolytic state in man. I. Induction and effects. J. clin. Invest. *38*, 1096–1109 (1959).
4 Gordon R. M., Crewe W.: The mechanism by which mosquitoes and tsetse flies obtain their blood meal, the histology of the lesions produced, and the subsequent reactions of the mammalian host; together with some observations on the feeding of *Chrysops* and *Cimex*. Ann. trop. Med. Parasit. *42*, 334–359 (1948).
5 Itard J., Jordan A. M.: Mass rearing using animals for feeding. In: Tsetse: The future for biological methods in integrated control (ed. by M. Laird), p. 125–140. IDRC, Ottawa 1977.
6 Jellison W. L., Kohls G. M.: Tick-host anemia: A secondary anemia induced by *Dermacentor andersoni* Stiles. J. Parasit. *24*, 143–154 (1937).
7 Lee R. I., White P. D.: A clinical study of the coagulation time of blood. Amer. J. med. Sci. *145*, 495–503 (1913).
8 Lester H. M. O., Lloyd L.: Notes on the process of digestion in tsetse flies. Bull. ent. Res. *19*, 39–60 (1928).
9 Nash T. A. M., Kernaghan R. J., Wright A. I.: A method for the prevention of skin reactions in goats used for feeding tsetse flies, *Glossina* spp. Ann. trop. Med. Parasit. *59*, 88–94 (1965).
10 Nash T. A. M., Jordan A. M., Trewern M. A.: Mass rearing of tsetse flies (*Glossina* spp.). In: Sterility principle for insect control or eradication, p. 99–110. I. A. E. A., Vienna 1971.
11 Nelson, W. A., Shemanchuk J. A., Haufe W. O.: *Haematopinus eurysternus:* Blood of cattle infested with the short-nosed cattle louse. Exp. Parasit. *28*, 263–271 (1970).

12 Nelson W. A., Bell J. F., Clifford C. M., Keirans J. E.: Interaction of ectoparasites with their hosts. Review article. J. med. Entomol. *13*, 389–428 (1977).
13 Parker K. R.: The interactions between *Glossina morsitans morsitans* and host rabbits: A host/biting-fly model. M. Sc. Thesis, University of Alberta, Edmonton, Alberta 1978.
14 Scarborough R. A.: The blood picture of normal laboratory animals. A compilation of published data. Yale J. biol. Med. *3*, 64–80 (1931).
15 Seebeck R. M., Springell P. H., O'Kelly J. C.: Alterations in host metabolism by the specific and anorectic effects of the cattle tick (*Boophilus microplus)* I. Food intake and body weight growth. Aust. J. biol. Sci. *24*, 373–380 (1971).
16 Seegers W. H.: Blood clotting enzymology. Academic Press, New York 1967.
17 Spector W. S.: Handbook of biological data. W. B. Saunders, Philadelphia 1956.
18 Steelman C. D.: Effects of external and internal arthropod parasites on domestic livestock production. Ann. Rev. Entomol. *21*, 155–178 (1976).
19 Van Handel E.: Coronary thrombosis and insect bites. Lancet *1962*/II, 886–887.