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Colonization of *Trypanosoma brucei gambiense* within transplanted Ehrlich's tumors of *Microtus montanus*

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Summary

Trypanosoma brucei gambiense has been observed growing in extravascular sites throughout a solid Ehrlich ascites tumor (EAT) in *Microtus montanus*. These trypanosomes appear in clusters in the tumor. The possible importance of this observation to the host-parasite relationship is discussed.

Key words: colonies; *Trypanosoma brucei gambiense; Microtus montanus;* extravascular sites.

There are recent reports which demonstrate that *Trypanosoma congolense* and *T. brucei* are located in extravascular sites in chronically infected animals (Luckins and Gray, 1978; Ssenyonga and Adam, 1975). In this paper, we present evidence for the presence of extravascular colonization by *T. b. gambiense* within transplanted Ehrlich's tumor of chronically infected *Microtus montanus*. In many ways, these colonies in vivo resembled the uneven distribution (or clouds) of trypanosomes growing over and between feeder-layer cells in vitro (Hirumi et al., 1977).

Materials and methods

Microtus montanus were obtained from an outbred colony maintained at Texas A & M University in College Station, Texas and maintained as previously described (Seed and Negus, 1970). All animals used were sexually mature and maintained on a 12 hour light-12 hour dark photoperiod regime. The maintenance and harvesting of both the trypanosome and the Ehrlich-ascites tumor cell (EATC) line have been described (Ackerman and Seed, 1976). Both cell types were washed in glucose (1%) phosphate buffered saline (pH 7.4) and then counted with the aid of a hemacytometer.

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The *Microtus* were infected intraperitoneally with 5×10^5 organisms of the Wellcome TS strain (TTrT-1) of *T.b.* The pathogenicity of this strain in *Microtus* has been described (Seed and Negus, 1970). Twelve days later, the infected animals were inoculated with 2×10^7 EATC subcutaneously; and sacrificed 8 days later.

The solid Ehrlich ascites tumors (EAT) were then removed and fixed in 1.25% (v/v) glutaraldehyde plus 4% sucrose in 0.05 M phosphate buffer. The tissues were later post-fixed in 1% buffered osmium tetroxide, dehydrated, and embedded in Epon according to standard procedures. Thick sections were prepared for light microscopy and examined after staining with 1% toluidine blue for 15 min at 45° C. Thin sections were cut and doubly stained with uranyl acetate and lead citrate. These preparations were examined with a Siemens 101 TEM.

Results and discussion

The light micrograph in Fig. 1 shows an extravascular concentration of trypanosomes within a solid Ehrlich tumor. The trypanosomes appeared to be distributed in clusters throughout the section. These clusters varied in both their size and location within the tumor, and were often a considerable distance from the vascular bed. In addition, there was no obvious correlation between the location of the clusters and the type of host or tumor cell present.

Similar observations were made using the transmission electron microscope. The electron micrograph also shows that the trypanosomes within the cluster appear morphologically intact with respect to their surface coat, cell membrane, and other organelles (Fig. 2). The trypanosomes within the tumors do not resemble published reports of either trypsinized or lysed cells, and dividing forms were observed within the tumors.

It is apparent that *T. b. gambiense* grow predominantly in extravascular sites throughout a solid EAT. We suggest that this may be a good model for further studies on the biology of trypanosomes in these sites.

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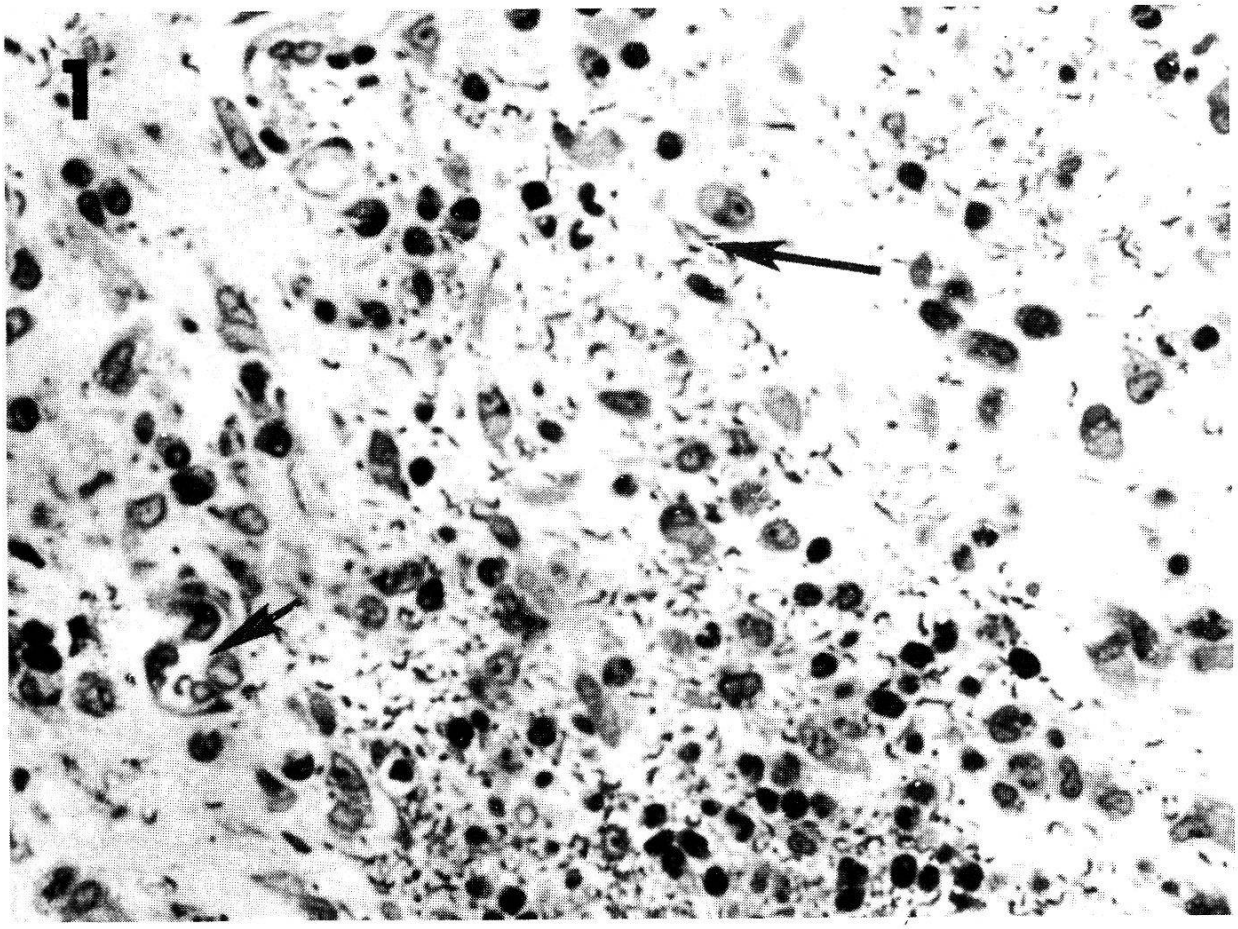
Fig. 1. A light micrograph (~320×) of a solid EAT from an infected *Microtus*. The long arrow points to one of many trypanosomes observed within the tissue section. The short arrow points to one of several small capillaries within the section.

Fig. 2. A transmission electron micrograph (~19000×) of trypanosomes within a solid EAT.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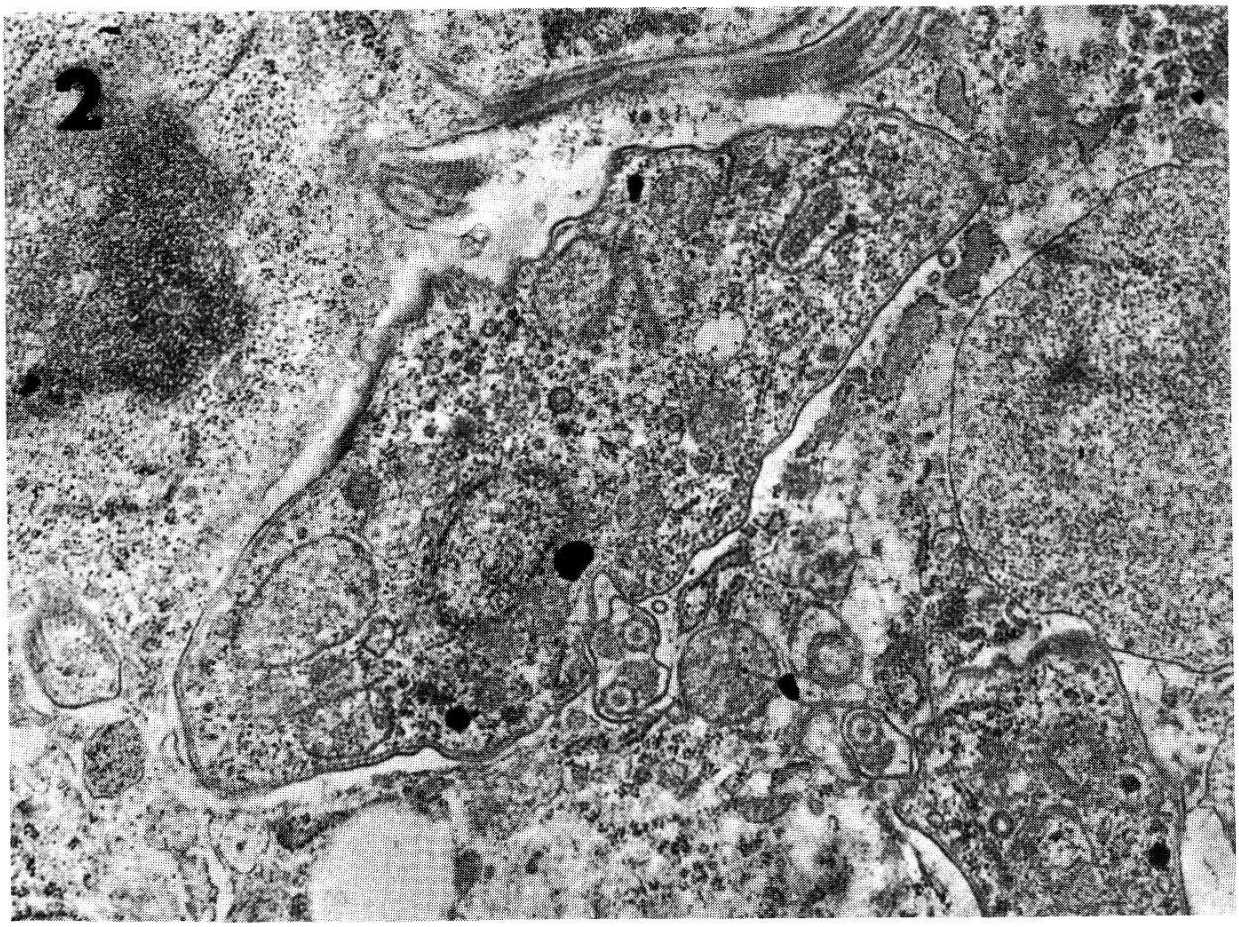
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