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Infectivity of *Leishmania donovani* primary culture promastigotes for golden hamsters

Short communication

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Leishmania donovani multiplies as an intracellular amastigote within macrophages of its host and as an extracellular promastigote within the midgut of the sandfly vector or when cultured in vitro. Within the alimentary tract of the sandfly, promastigotes undergo a sequence of morphological and presumably physiological changes (Killick-Kendrick, 1979) which have not yet been demonstrated for culture forms. Although there are seldom more than 10 parasites in the probosces of even heavily infected flies, these few are capable of producing infections in experimental hosts and man. Promastigotes cultured in vitro, however, show greatly reduced infectivity for experimental hosts, and may become noninfective after extensive subculturing (Giannini, 1974). Despite some evidence that promastigotes from young cultures are less infective for hamsters than those from older cultures (Giannini, 1974), there have been no studies in which animals of the same age, sex, and weight were given a standard inoculum of primary culture promastigotes (PCP) from consecutive days in culture to see what effect culture age and cell type might have on infectivity. In order to evaluate more precisely the infectivity of *L. donovani* 3 through 12-day old PCP for hamsters, intracardial injections of promastigotes were combined with an 8-day screening technique (Stauber et al., 1958), the number of parasites in liver impression smears were counted, and cell types in culture were checked daily by direct observation of smears. Our data show that log and stationary phase PCP behave as a continuously infective population for hamsters and that infectivity is independent of morphological type.

Amastigotes of *L. donovani* Sudan strain 1S (Stauber, 1966a) were obtained aseptically from heavily infected spleens of randomly-bred golden hamsters, *Mesocricetus auratus* (LVG:LAK, LAK, River, Wilmington, Massachusetts), which had been inoculated intracardially with 0.1 ml spleen suspen-

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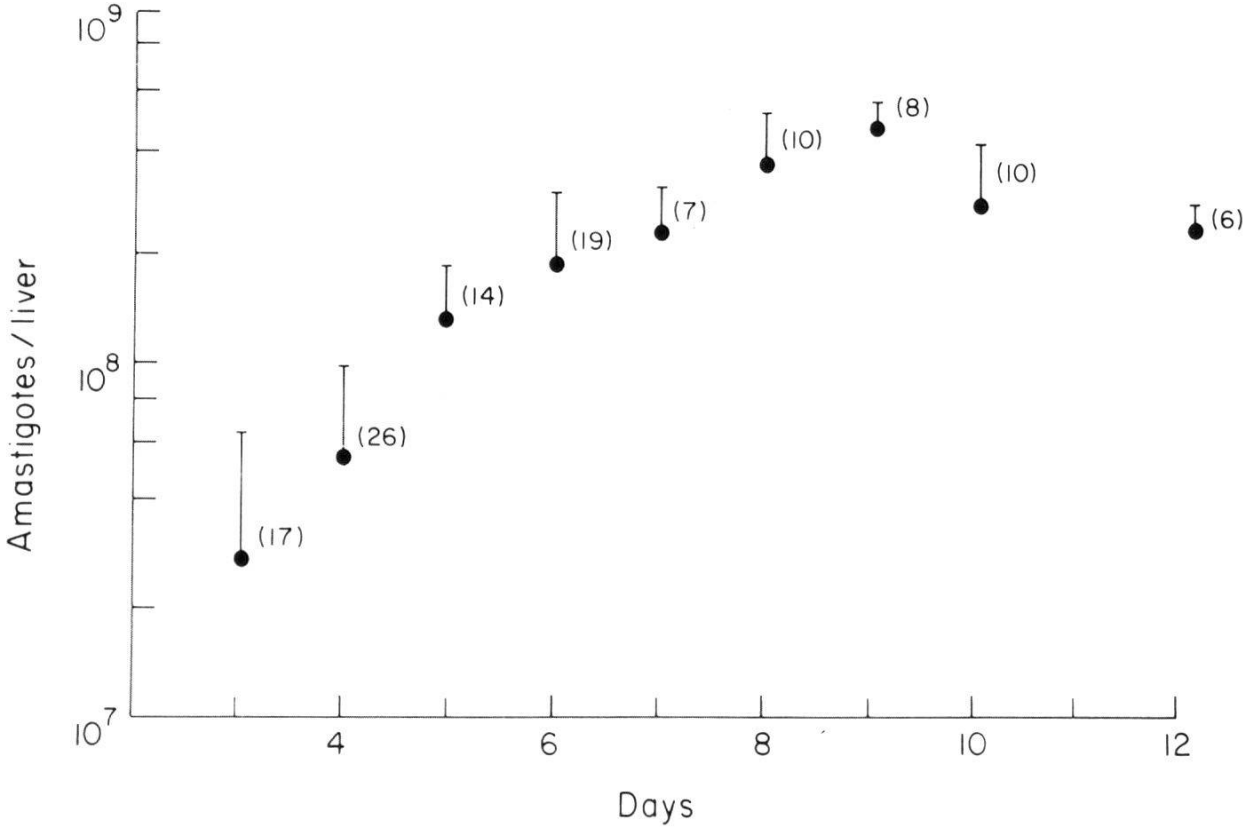

Fig. 1. Infectivity of *Leishmania donovani* PCP for hamsters as measured by number of amastigotes in the liver at 8 days. Bars = ±SD, (n) = number of hamsters.

sion containing 20–80 million amastigotes 4 to 6 weeks earlier. Spleens were weighed, homogenized and centrifuged as previously described (Keithly, 1976). Amastigotes were counted in a Neubauer hemocytometer and were diluted with HBSS to give a final suspension of 20×10^6 parasites/0.1 ml for inoculation into blood agar tubes overlaid with HBSS (Tobie et al., 1950). Growth of *L. donovani* promastigotes in this medium was determined by removing a 0.1 ml sample from each of 3–5 randomly chosen tubes and diluting 1/10 with formalin (35% w/v): HBSS. Cell number was recorded every 3 h for the first 2 days, and every 12 h thereafter for a total of 12 days. Daily, one of these tubes and an uninoculated control tube were shaken to disperse the overlay, opened, and the pH of each overlay directly measured with a combination glass electrode.

In six separate experiments, each of 6 to 10 outbred male hamsters (weight as observed, 96.8 g ± 11 [mean ± SD of 127 total animals]) were anesthetized with Nembutal (Abbott Laboratories, Chicago, Illinois) according to body weight, and were inoculated intracardially with 0.1 ml HBSS containing 30 million PCP harvested from days 3 through 12. Except for days 3, 10 and 12, at least three experiments for each culture period were made. Before and after injection, duplicate smears of the inoculum were prepared. Types were designated by shape as ovals, flagellated ovals, stumpys, short slenders, and long slenders. An analysis of the variation in PCP morphological type was made by counting at least 500 parasites from each smear, and the percent of each type

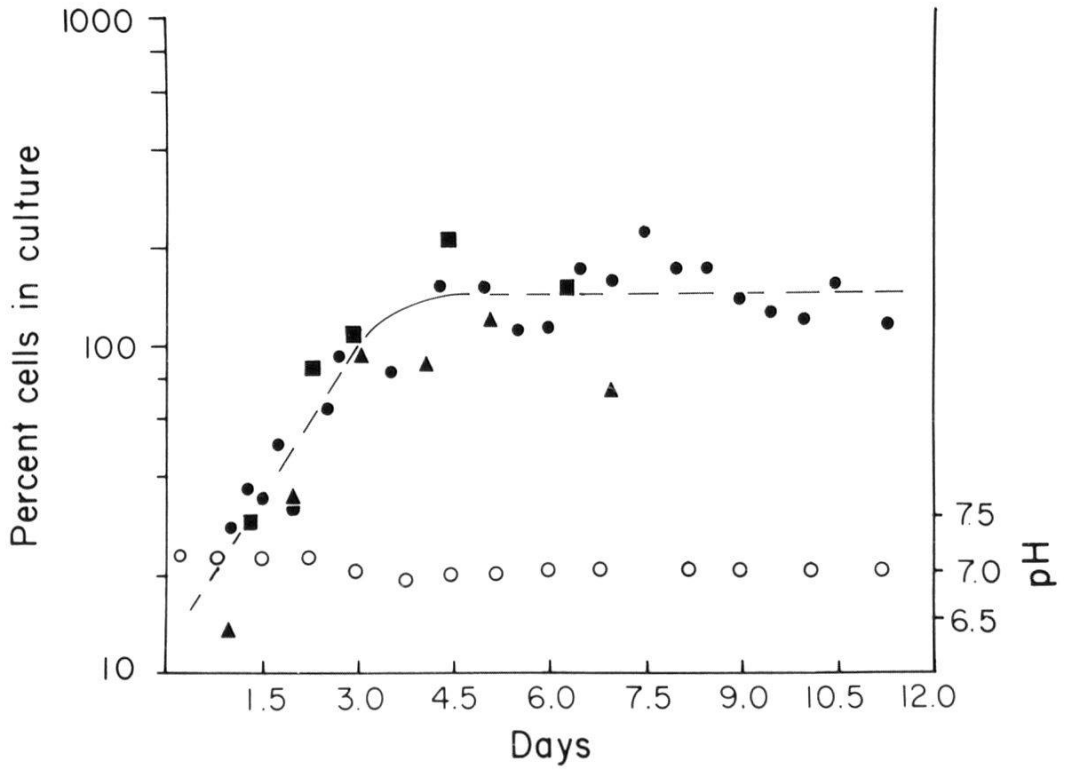

Fig. 2. Growth of *Leishmania donovani* in Tobie's medium. The relative number of cells is normalized at 3 days to 100%. The data from this experiment ●, are plotted with those from two (unpublished) experiments ■, ▲, performed several years previously. ○ = pH of the overlay of the cultures.

calculated from these data. Infectivity was determined at necropsy 8 days after infection as described before (Keithly, 1976). The Student-Newman-Keuls analysis of variance and multiple range tests were used to determine the significance of values obtained ($p < 0.01$).

At all time points tested, *L. donovani* PCP were infective for hamsters. Infectivity decreased as expected after initial transformation from amastigote to promastigote (Keithly, 1976), and thereafter increased exponentially from day 3 through day 9 (Fig. 1). At the 1% significance level, promastigotes from culture day 9 had significantly higher, and those from culture days 3 and 4 had significantly lower infectivity for hamsters than did promastigotes from other days. Infectivity decreased slightly after day 9.

Morphological analysis of cultures showed that all types undergo division and that the frequency of the different types varies somewhat from days 3 through 12. Although short slender forms were predominant (>50% of population) throughout the experiment, their relative contribution to the population changed <20%, a change which could barely explain the one log increase in infectivity over the same time period. None of the other morphological types show more than a twofold increase in their relative contribution to the cell population at any time. Since the change in infectivity of PCP for hamsters during this testing period is one log number (Fig. 1), it would be necessary to demonstrate at least a tenfold increase in one or another type in order to directly

attribute any difference in infectivity to a change in cell morphology. Therefore, our study strongly suggests that changes in infectivity cannot be correlated with morphological type. Analysis of growth (Fig. 2) shows there are lag (0–1 days), log (1–3.5 days), and stationary (3.5–11 days) phases. Transformation of *L. donovani* amastigotes into promastigotes occurs during lag phase, and cells begin to divide between 18 and 24 h (generation time $\cong$ 21 h). These observations agree essentially with those of other workers (Chang and Negerbon, 1947; Christophers et al., 1926; Janovy, 1967; Simpson, 1968). In both uninoculated and inoculated tubes, there is a slight initial drop in pH (about 0.4 and 0.3 units, respectively), but no significant further changes are observed for the duration of the experiments. Therefore, unlike other hemoflagellates (Chang, 1948), *L. donovani* PCP do not produce marked changes in the pH of the medium which might influence their infectivity for hamsters.

These data reaffirm the utility of intracardial infection and 8-day liver impression smears as a rapid, reproducible method for assaying the infectivity of *L. donovani* for hamsters; provided that animals of the same age, weight, and sex are used, and that sufficient numbers of promastigotes are injected. They also confirm preliminary results which showed that 4 to 7-day old PCP of *L. donovani* behave as a single, continuously infective population for hamsters (Keithly, 1976), and extend these observations to show that there is a log change in infectivity of 3 to 12-day old PCP for hamsters which is related to culture age, but which is independent of cell morphology, pH of medium, or growth phase in culture.

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