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MACROPHAGE-TRYPANOSOME INFECTION. DEAD AND LIVE CELL SCORING (*)

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INTRODUCTION

During the course of our experiments on the interaction between peritoneal macrophages and **Trypanosoma cruzi** in vitro, we were interested to observe the effect of the parasite on the host cell. Using the trypan blue dye exclusion test for viability we were faced with the technical problem of visualizing trypanosomes within cells in light microscopy, without staining, the only way to distinguish live from dead cells by this method.

We here describe a modification of the trypan blue dye exclusion test which overcomes this difficulty.

Macrophages harvested from the peritoneum of peptone stimulated mice were allowed to settle on coverslips, and incubated for 24 hours at 35°C in tissue culture medium (M199, Flow Lab.. U.K.) with 20% fetal calf serum. Trypomastigotes of **T. cruzi** were obtained from the supernatant of LLC-MK_2 tissue culture infected cells, and kept in contact with macrophage monolayers for 3 hours. Monolayers were washed and re-incubated in M 199 for 96 hours, at 35°C.

Observations were carried out, washing coverslips in pre-heated phosphate buffered saline (PBS) and exposing them for 5 minutes to a 0.2% trypan blue solution in PBS. At this stage live cells could be distinguished from dead ones, but parasites were hardly visible. Subsequently cells were washed in PBS, fixed for 5 minutes in Bouin's solution, and washed repeatedly in 80% ethylic alcohol and further in 100% alcohol, xylene, and finally mounted on slides.

After processing coverslips as mentioned above, live and dead cells could be scored, as well as infected and non-infected cells, since during fixation the blue staining of dead cells maintained itself while parasites revealed their outline very distinctly.

RESUMO

Reconhecimento de células viáveis na infecção de macrófagos por tripanosomas

Descreve-se uma técnica, usando azul de tripano e posterior fixação, que permite visualização perfeita de **T. cruzi** no interior de macrófagos e ao mesmo tempo a distinção entre células hospedeiras vivas e mortas.

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