Active penetration of *Trypanosoma cruzi* into host cells: historical considerations and current concepts

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INTRODUCTION AND EARLY STUDIES
Investigation of the interactions of *Trypanosoma cruzi* with host cells became possible after techniques to cultivate this protozoan in tissue culture were developed. The first approach was described by Kofoid et al. (1935), who showed that the protozoan could survive and multiply in cultures of heart cells from mouse and rat embryos. A subsequent study by Romana and Meyer (1942) using chick embryo heart cell cultures described the behavior of *T. cruzi* in tissue cultures in detail. Theirs in vitro observations of the interaction process in both living and fixed cultures led the authors to describe two mechanisms of cell infection by the protozoan: active penetration and phagocytosis. In this classic paper, it was stated that “in general, the active penetration was more visible with metacyclic forms that, with their great motility, easily crossed the cell surface, penetrating into the protoplasm of fibroblasts and myocytes.” To the best of our knowledge, this is the first reference to the active penetration of parasitic protozoans into cells. Six years later, Meyer and Xavier de Oliveira (1948) published a paper and a small book confirming the initial observations and reported that the parasite “can touch the surface of the host cells without penetration. Occasionally, they adhere to the cell surface and suddenly penetrate into the cell.” These observations were more clearly demonstrated in a classic film by H. Meyer and A. Barasa. Part of that film which is kept in our laboratory (founded in 1940 by H. Meyer) is available at the site of the Brazilian Society of Protozoology (www.sbpz.org.br). The same basic idea initially proposed by Meyer and colleagues was presented in 1973 by Dvorak and Hyde (Dvorak and Hyde, 1973) based on observations of the interaction of *T. cruzi* trypomastigotes of the Ernestina strain with secondary bovine embryo skeletal muscle cells (BESM) and HeLa cells obtained through phase contrast microscopy under controlled conditions. The process of cell invasion was described as an active penetration process in which mechanical activity of the protozoan is prevalent, and the parasites penetrate into the cell through the plasma membrane. According to these studies, the parasites enter cells posterior end first.

It is important to note, although it is not the focus of the present review, that the same basic idea of active penetration has also been applied to other protozoans, such as *Toxoplasma gondii* and *Plasmodium*, which were again analyzed first by Nery Guimarães and Meyer (1942) and Dvorak et al. (1973).

SECOND PHASE
One of the authors of this review (WS) joined the Hertha Meyer’s laboratory in 1969 and began the first studies analyzing the interaction of *T. cruzi* with chick embryo heart muscle cells and macrophages using electron microscopy. Jim Dvorak visited the laboratory several times between 1972 and 1980, and intense discussions about the concept of active penetration took place at that time. With enthusiasm, Jim defended the idea that *T. cruzi* and *Plasmodium* are able to generate a transient tunnel-like structure in the host cell plasma membrane that is sealed immediately after parasite internalization, and the parasite then establishes intimate contact with the host cell cytoplasm. Up to this point, electron microscopy had not been used to analyze the parasitic protozoan-host cell interaction process. However, a few images obtained in our laboratory showed that recently penetrated trypanomastigotes were not in contact with the myofibers of heart muscle cells but were instead located within a membrane-bound vacuole. These observations were made approximately in 1972.
We had the opportunity to discuss the data described above as *T. cruzi* by 
*a* vacuole, designated the parasitophorous vacuole (PV) following 
host cell. The parasite is able to stimulate endocytic activity in the future 
induced phagocytosis has been used to refer to a process in which 
protozoa. The same basic idea can be extended to other intracellular parasitic 
tions clearly indicate that an endocytic process is always involved 
complete disappearance of the PV. Taken together, these observa-
tions, clearly show that a parasitophorous vacuole always pene-
trate the host cell in a process that is better characterized as an 
endocytic process, involving the initial formation of an PV. This process takes place independent of the nature of the host cell. 
Even so-called non-professional phagocytic cells can be penetrated by 
*T. cruzi* and *T. gondii*. The term induced endocytosis or even 
induced phagocytosis has been used to refer to a process in which the 
parasite is able to stimulate endocytic activity in the future host cell. 
According to Nogueira and Cohn in the case of *T. cruzi*, sub-
sequent to penetration, the parasites leave the PV in a process they described as "escaping" and then enter into direct contact with the host cell cytoplasm. However, a few years later, it was shown (Carvalho and de Souza, 1989) that there is no escaping, but rather, fragmentation of the PV membrane occurs, most likely due to the activity of enzymes secreted by the parasite, as was shown (Carvalho and de Souza, 1989) that there is no escaping, but rather, fragmentation of the PV membrane occurs, most likely due to the activity of enzymes secreted by the parasite, as was 
recently shown by Andrews et al. (1990), associated with the 
complete appearance of the PV. Taken together, these observa-
tions clearly indicate that an endocytic process is always involved 
during the process of *T. cruzi* internalization into a host cell. The 
same basic idea can be extended to other intracellular parasitic 
protozoa. 

**A NEW CONCEPT OF “ACTIVE PENETRATION”**

We had the opportunity to discuss the data described above as well as the results obtained by several groups in *Plasmodium* and *Toxoplasma* with Hertha Meyer, James Dvorak, Nadia Nogueira, and Zavvil Cohn, and we reached the conclusion that active penetration, as initially defined, does not exist during the process of parasitic protozoa interacting with host cells. A possible excep-
tion is observed during infection of host cells by microsporidia, 
which include a large number of species initially considered to 
be protozoans but that have more recently been considered to be fungi based on the presence of a large number of genes that, upon phylogenetic analysis, cluster Microsporida with Fungi (see review 
by Xu and Weiss, 2005). These organisms present a complex life 
cycle, and their spores contain a structure known as the polar 
tube and is transferred to the host cell cytoplasm, where it will 
bequeathed to the host cell in a process that is better characterized as an 
endocytic process, involving the initial formation of an PV. This process takes place independent of the nature of the host cell. 
Even so-called non-professional phagocytic cells can be penetrated by 
*T. cruzi* and *T. gondii*. The term induced endocytosis or even 
induced phagocytosis has been used to refer to a process in which the 
parasite is able to stimulate endocytic activity in the future host cell. This 
penetration could be used to indicate the fact that the parasite 
will develop.

After some discussion, it was concluded that the term active penetration could be used to indicate the fact that the parasite 
plays an important role in the "induction of the host cell invasion 
process." Indeed, since the first description of the process, it has 
been clear that the intense movement of the protozoan, especially 
due to the flagellar beating process, plays some role. This idea was 
further analyzed by Schenkman et al. (1991), who showed that 
maintenance of an active energetic metabolism is fundamental for 
*T. cruzi* to invade cells, as this process is prevented by treatment 
of the parasites with 2-deoxy-glucose, an inhibitor of glycolysis, 
as well as sodium azide, antimycin, and oligomycin, which interfere 
with the mitochondrial metabolism involved in the synthesis of ATP. 

The term active penetration has also been employed from another perspective. Kipnis et al. (1979), for example, used it 
to describe the penetration of bloodstream trypanosomatids into 
macrophages in a process that was only partially inhibited by 
cytoschalin B. At present, we know that this compound does not inhibit all forms of endocytosis. 

Therefore, we can conclude that the available data, especially 
those obtained through transmission electron microscopy of thin 
sections, clearly show that recently penetrated *T. cruzi* of both infective and non-infective forms are always located within a PV 
that interacts with the organelles of the endosomal–lysosomal sys-
tem during its short existence. This phenomenon occurs in all 
cell types examined to date independent of whether they are 
professional or non-professional phagocytic cells. The formation 
of the PV involves the induction of a calcium flux into the host cell 
via the action of a parasite-derived calcium agonist, which is 
generated through the action of a parasitic oligopeptidase (Caler 
et al., 1998), as well as the synaptotagmin VII pathway (Caler 
et al., 2001), the recruitment of lysosomes (Tardieu et al., 1992), 
and the participation of microtubules (Tyler et al., 2003) and 
actin filaments (Cordero et al., 2002). Recently, Fernandes et al. 
(2011) showed that *T. cruzi* trypanosomatids mimic the process of 
wound repair with Ca2+-dependent exocytosis of lysosomes by 
delivering acid sphingomyelinase to the host plasma mem-
brane, facilitating parasite entry into host cells. These aspects of 
the invasion process have been extensively reviewed in recent years 
(Hall, 1993; Burleigh and Andrews, 1995; Yoshida, 2006; Alves 
and Collis, 2007; de Souza et al., 2010; Caradonna and Burleigh, 2011; 
Butler and Tyler, 2012; Fernandes and Andrews, 2012; Romano 
et al., 2012). 

**ENDOCYTOSIS IS A COMPLEX BIOLOGICAL PROCESS**

Our present knowledge of the endocytic process shows that it 
is more complex than previously thought. Indeed, in addition to 
the classical phagocytic process, there are several ways a cell can 
ingest extracellular material of variable dimensions. These mech-
анisms can be either dependent on dynamin, such as the clathrin- 
and caveolin-mediated processes, or independent of dynamin, as 
occur during processes including macropinocytosis, and lipid
raft-mediated endocytosis. It is likely that other mechanisms will be described in view of the large number of groups attempting to better characterize the endocytic process. As described in another review in this volume (Barrias et al., submitted), T. cruzi may use all of these mechanisms to enter host cells. It is possible that the parasite selects the mechanism to be used based on factors such as the nature of the cell and the host cell surface ligand to which

**ACKNOWLEDGMENTS**

The authors wish to thank the support of CNPq, CAPES, and FAPERJ.

**Conflict of Interest Statement**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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