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TRYPANOSOMA CRUZI: VERTEBRATE AND INVERTEBRATE CYCLES IN THE SAME MAMMAL HOST, THE OPOSSUM *DIDELPHIS MARSUPIALIS*

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Epimastigotes multiplying extracellularly and metacyclic trypomastigotes, stages that correspond to the cycle of Trypanosoma cruzi *in the intestinal lumen of its insect vector, were consistently found in the lumen of the anal glands of opossums* Didelphis marsupialis *inoculated subcutaneously with infective feces of triatomid bugs.*

Most species of Protozoa of the genus *Trypanosoma* are digenetic parasites whose complete life-cycle involves two alternate hosts, a vertebrate and an invertebrate that functions as vector. For *Trypanosoma cruzi,* the causative agent of Chagas's disease in man, the vertebrate hosts are numerous species of mammals of 9 orders and the vectors are haematophagous insects distributed in several genera of the sub-family Triatominae (Hemiptera, Reduviidae).

In the vertebrate host, *T.cruzi* is typically an endocytic parasite which, in the amastigote stage, multiplies by binary fission in a variety of cells, while in the insect its habitat is the lumen of the digestive tract where the parasite mutiplies (extra-cellularly) as epimastigote. The parasite does not divide as trypomastigote; in the vertebrate this stage is produced at the end of the intracellular multiplication cycle and invades new cells or, passing into the bloodstream, transfers the infection to the invertebrate; in this host the trypomastigote ("metacyclic") is also the end product of multiplication cycles and is the infective form for mammals. Both the vertebrate and invertebrate cycles can be imitated "in vitro", the first in cell cultures maintained at temperatures of 33^0C or above, the latter in anexic culture media at temperatures of $27–28^0$C. The organism of a mammal is normally entirely inhospitable to the stages that multiply in the insect (or in axenic cultures) and not only because of the high body temperature: epimastigotes (but not the metacyclic trypomastigotes) are rapidly lysed by complement or endocytized and digested by phagocytes.

Marsupials of the genus *Didelphis,* common throughout temperate and tropical areas of the Americas, are thought to be important wild reservoirs of *T. cruzi,* with high rates of natural infection.

In our studies of the experimental infection in the species *Didelphis marsupialis* a number of these animals were inoculated with various strains of the parasite and followed for periods that now amount to almost three years for some of them. It was seen that since early age the opossum survives inocula that kill mice within a short time. Two types of infection are produced: 1) apparently self-limited, results of sub-inoculation (in mice), xenodiagnosis and hemoculture becoming consistently negative within few weeks post-inoculation; immunofluorescent antibody test (IFAT) positive at low titers; 2) the parasite regularly or, more often, intermittently recuperated through xenodiagnosis and/or hemoculture after many months; IFAT persistently positive in high titers (Deane, Jansen & Mangia, 1983; Jansen et al., 1984). These differences are related to the strain of *T. cruzi* and we have evidence that the parasite may persist in the organism of the opossum even when the infection is of the first type. However, in all cases the intracellular forms are extremely hard to find in necropsied animals, in the tissues which usually shelter *T. cruzi* in infections of man and other mammals. The question then arose: "Where does the opossum conceal the parasite?"

Following a systematic search we have found the parasite in great numbers in sections of the anal glands of two animals that had died four and six months after being subcutaneously inoculated with *T. cruzi.* Other opossums that had been similarly infected were killed and, in fresh preparations made with material collected directly from the lumen of those glands, large masses of motile epimastigotes and free, very active trypomastigotes of the metacyclic type were found. Morphology of the flagellate in fresh and Giemsa stained films and imprints was characteristic of *T. cruzi* in the stages found in the invertebrate host and in axenic cultures (Fig. 1). This material has also been inoculated in mice and opossum, fed through a membrane to triatomid bugs and passaged in culture media: infections and growth in culture, as well as morphology of the parasites recuperated are identical with *T. cruzi.*

So far the anal glands have been found infected in six out of eight opossums in which they have been examined. These animals, three males and five females, were part of two litters found in the pouch

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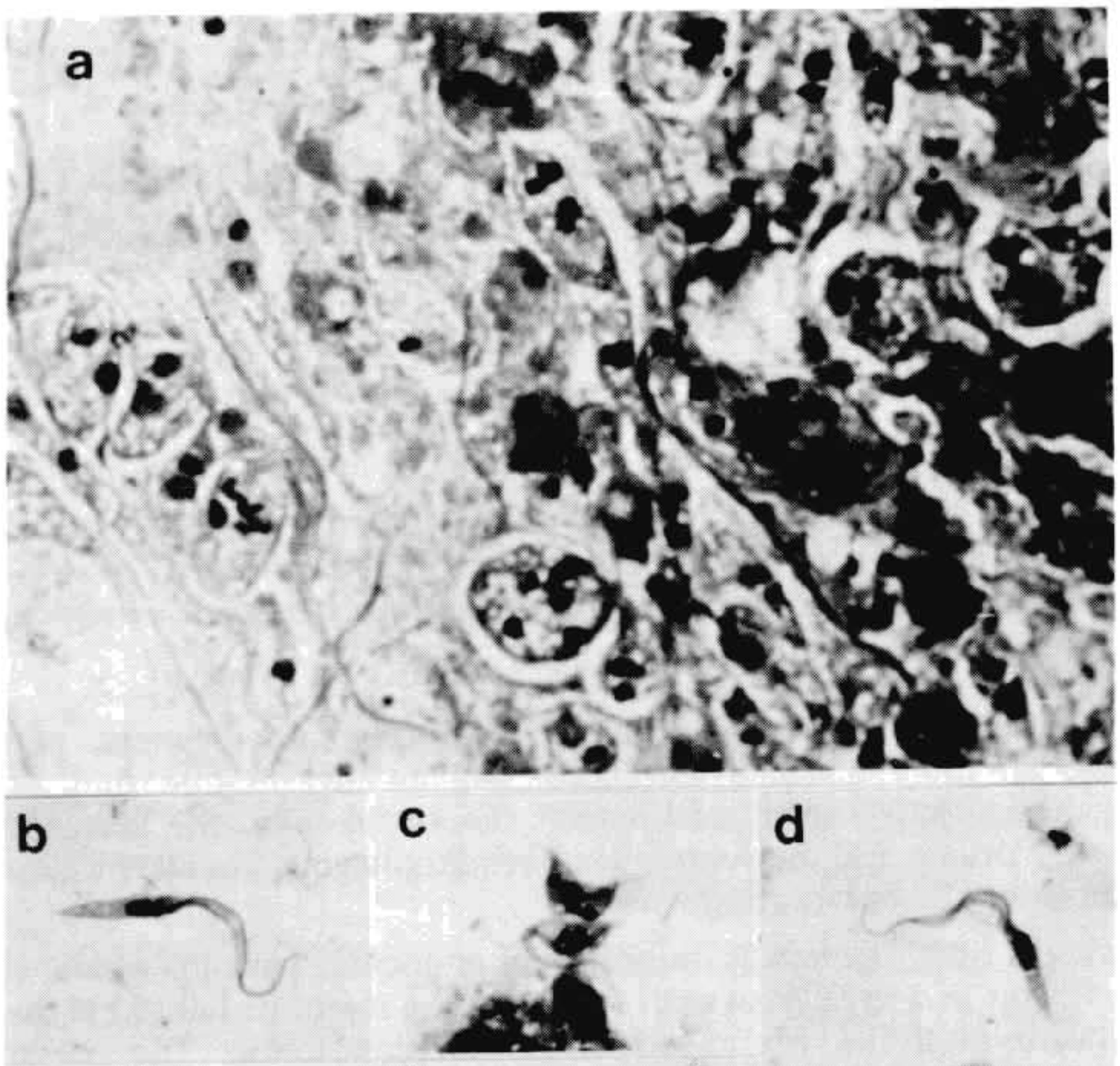

Fig. 1: Giemsa stained preparations of the material in the lumen of the anal glands of opossums previously inoculated subcutaneously, in the dorsal aspect of the thorax, with metacyclic trypomastigotes of *Trypanosoma cruzi*. *a:* inprint showing a mass of parasites among some isolated epithelial cells and a coarse, amorphous material (X 2000); *b* to *i* (X 1600): developmental forms of the parasite in thin films made from Hank's saline suspension of the glands secretion; typical epimastigotes, some in division, cytoplasma full of inclusions possibly lipids; *g:* a transforming stage; *h* and *i:* metacyclic trypomastigotes with the conspicuous kinetoplast characteristic of *T. cruzi*.

of trapped females which were repeatedly negative for *T. cruzi* through xenodiagnosis, hemoculture and serology. The estimated age of the litters at capture was 10 and 30 days and they were inoculated while still in the marsupium, at about 88 and 80 days of age, respectively, with metacyclic trypomastigotes from triatomid bugs infected with strains "G-N" and "G-49" of *T. cruzi*, both isolated by xenodiagnosis from wild *D. marsupialis*. In the whole group parasitemia was patent within the first week and remained so for a maximum of 12 weeks thereafter; all the animals except one had repeatedly positive xenodiagnoses and hemocultures and all maintained IFAT at titers above 1:80 and up to 1:640, to the date of the necropsy. As it is customary in *T. cruzi* infections, intracellular forms seem to be very scarce in the tissues of these opossums. However, the trypomastigotes found in the peripheral blood during the acute parasitemia were of the broad bloodstream type and the whole picture indicates the usual sequence of a systemic infection. Therefore, it is concluded that *T. cruzi* was performing the vertebrate as well as the invertebrate cycle in these opossums.

A somewhat similar situation has been reported before: in embryonated chicken eggs inoculated with *T. cruzi* bloodstream trypomastigotes, Mello & Deane (1976) found both the vertebrate and invertebrate cycles going on, side by side, the first in the embryo itself and its membranes, the latter in the egg cavities, especially the yolk sac, when the infected eggs were reincubated at a temperature of 32-34°C. In this case, temperature was an important factor, since identical inocula produced only stages of

the vertebrate cycle when the incubation temperature was maintained above 37°C. It may be relevant to mention that the average body temperature, reported for opossums (Hunsaker, 1977) is well below that of eutherian mammals.

The glands found infected with *T. cruzi* in our opossums contained a dark brown sticky, waxy material, difficult to suspend in saline. It has been for us quite a surprise to find the flagellate immersed so abundantly in such a coarse and semi-solid environment. It is evident that *T. cruzi* epimastigotes find in it what nutrients they need. The material is apparently rich in lipids and in Giemsa stained preparations the flagellate appears full of inclusions that are possibly of a lipidic nature (see Fig. 1). It has been reported (Oliveira, Timm & Costa, 1977) that lipids make up for 15% of *T. cruzi* dry weight (culture forms). No parasites were detected within the stratified epithelium or the striated muscular layer that surround the gland.

The localization of the parasite in the anal glands of the opossum suggests a very high degree of adaptation. It may be speculated that, besides the nutrients, these glands afford: 1) a reservoir from which the parasite may be disseminated in the tissues and bloodstream of the host and thus be within reach of the hematophagous vector; 2) a very efficient protection against the immune response of the host. We have demonstrated that the opossum is a good responder to *T. cruzi* antigens and able to control highly virulent inocula as soon as it outgrows early embryonic development. As mentioned, the specimens found infected in the glands had high antibody titers detected by IFAT. We have also found that normal opossum serum lyses *T. cruzi* culture epimastigotes as rapidly as does human or guinea-pig serum (Thomaz & Deane, unpublished). It is evident that, within these exocrine glands the parasite escapes adverse factors both inespecific and immune, that circulate in the host organism.

This new finding, which is reproducible and not fortuitous, may offer some answers and surely suggests a wealth of relevant questions about the relationship between *T. cruzi* (and other trypanossomatidae?) and its many vertebrate hosts.

RESUMO

No gambá *(Didelphis marsupialis)* foi observado um ciclo extracelular do *Trypanosoma cruzi:* o parasita crescia abundantemente no material de secreção acumulado no lúmen das glândulas anais de animais criados em cativeiro e infectados por via subcutânea com fezes de triatomíneos.

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