Japan. J. Trop. Med. Hyg., Vol. 15, No. 2, 1987, pp. 97-104

THE FATE OF *LEISHMANIA BRAZILIENSIS, L. DONOVANI* AND *TRYPANOSOMA CRUZI* IN DIFFUSION CHAMBERS IMPLANTED INTO HAMSTERS AND MICE —— A PRELIMINARY STUDY ——

YOSHIHISA HASHIGUCHI[1], MASATO FURUYA[2] AND YOSHISUKE OKAMURA[1]

Received January 22 1987/Accepted March 30 1987

Abstract: *Leishmania braziliensis* and *L. donovani* were investigated for the transformation and survival in intraperitoneal (IP), subcutaneous (SC) and intrascrotal (IS) diffusion chambers implanted into hamsters and mice. For a comparison, *Trypanosoma cruzi* was also examined by using the same procedure. The 2 *Leishmania* species revealed an unexpectedly short survival time, and no transformation was observed in the parasites in chambers implanted into hamsters or mice. IS chambers seemed to provide a better condition for *L. donovani, L. braziliensis* and *T. cruzi,* as compared with IP and SC chambers in hamsters. In the study, no IS chambers were examined in mice because of too small size of the scrotum to insert the diffusion chamber. *T. cruzi* showed a considerably longer period of survival than *L. donovani* or *L. braziliensis* in mice, but not in hamsters. The trypanosome, *T. cruzi,* transformed from epimastigote to trypomastigote and amastigote in IP and SC chambers in mice. These results seemed to suggest that the factors responsible for the transformation and survival of the organisms might be greatly different between the 2 genera, *Leishmania* and *Trypanosoma,* and also between the 2 host animals, hamsters and mice.

INTRODUCTION

The parasite of the genus *Leishmania* is an obligatory intracellular organism as amastigote in vertebrate hosts and extracellular one as promastigote in invertebrate hosts. Using such a protozoan, it seems to be interesting to make analysis *in vivo* on the interrelation between the parasite in chambers and the host animals, by implanting diffusion chambers which separate the parasite from a direct physical contact with various host cells by microporous filters. Providing that the organisms survive, and preferably transform and differentiate in the chambers implanted, they provide a useful model for investigating both biology of the parasite and development of immunity in the host. Diffusion chambers allow exchange of various diffusible substances between the parasites in chambers and their hosts. Moreover, if desired, the chamber may be easily retrieved for examination of the implanted organisms. On the basis of these advantages, diffusion chambers have been used to culture *in vivo* several species of parasites, and to immunize host animals against protozoans and helminths.

In *Trypanosoma cruzi,* morphogenesis of the parasite and development of the host immunity

1 Department of Parasitology, Kochi Medical School, Nankoku City, Kochi 781-51, Japan
2 Institute for Laboratory Animals, Kochi Medical School, Nankoku City, Kochi 781-51, Japan

have been studied by using diffusion chambers implanted intraperitoneally into mice (Logan and Hanson, 1974). Moreover, in subcutaneous diffusion chambers, *T. brucei, T. rhodesiense* and *T. gambiense* multiplied rapidly, persisted for as long as 5 weeks and expressed antigenic variation (Ballon-Landa *et al.*, 1985).

In contrast to the genus *Trypanosoma,* no such a study has been attempted in the genus *Leishmania.* In the present study, therefore, a preliminary trial was undertaken to determine whether diffusion chamber is suitable for obtaining information on the interrelation between the implanted parasites and the hosts. As the first step, the transformation and survival of *L. braziliensis* and *L. donovani* were examined in intraperitoneal, subcutaneous and intrascrotal chambers. In the experiment, for a comparison, *T. cruzi* was also examined by using the same procedure.

Materials and Methods

Host animals

Adult male BALB/c mice and Syrian hamsters, *Mesocricetus auratus,* weighing around 30 g and 130 g, respectively, were used. They were fed with a commercially prepared diet and water was provided *ad libitum.*

Parasites and the in vitro cultivation

The organisms studied were 3 species of protozoans, *L. braziliensis, L. donovani* and *T. cruzi.* The 2 species of *Leishmania* were kindly supplied by the Department of Parasitology, Keio University School of Medicine, Tokyo, Japan, and *T. cruzi* was obtained from a patient with Chagas' disease in Guayaquil City, Ecuador. The parasites were maintained at 26°C in a serial *in vitro* passage, as promastigote in *Leishmania* and epimastigote in *Trypanosoma,* in culture medium (Aljeboori, 1979); a part of *in vitro* culture was performed by using Pan's medium (Pan, 1984). No difference was recognized on the transformation and survival between the parasites derived from the 2 culture media used. All the materials used for inoculation of diffusion chambers were from stationary phase of the culture.

Diffusion chambers and the implantation or removal

Diffusion chambers were made of a plexglass ring (U-100, diameter: 10 mm, thickness: 2 mm) and millipore filters (pore size: 0.22 μm). The chambers were constracted by cementing filters to either side of a ring with MF cement (Millipore Filter Co.). Diffusion chambers were sterilized with U. V. for overnight in the experiment. These chambers were aseptically filled with 0.1 m*l* (3×10^5 to 1×10^6 cells) of the parasite suspension. After loading, the access hole of each chamber was sealed with a plastic plug and then covered with a drop of MF cement and allowed to dry before implantation.

For implantation of a diffusion chamber, the hair of the abdominal and scrotal regions of the animals was clipped with an electric clipper, the animals anaesthetized with Nembutal, and the clipped regions were washed with 70% ethanol. An incision about 1.5 cm long was made and then diffusion chamber containing parasites was placed intraperitoneally, subcutaneously and intrascrotally. The incision was closed with silk sutures.

At the removal and examination of these chambers, the animals were anaesthetized and surgically opened, and the chambers were removed and examined for the parasites at intervals.

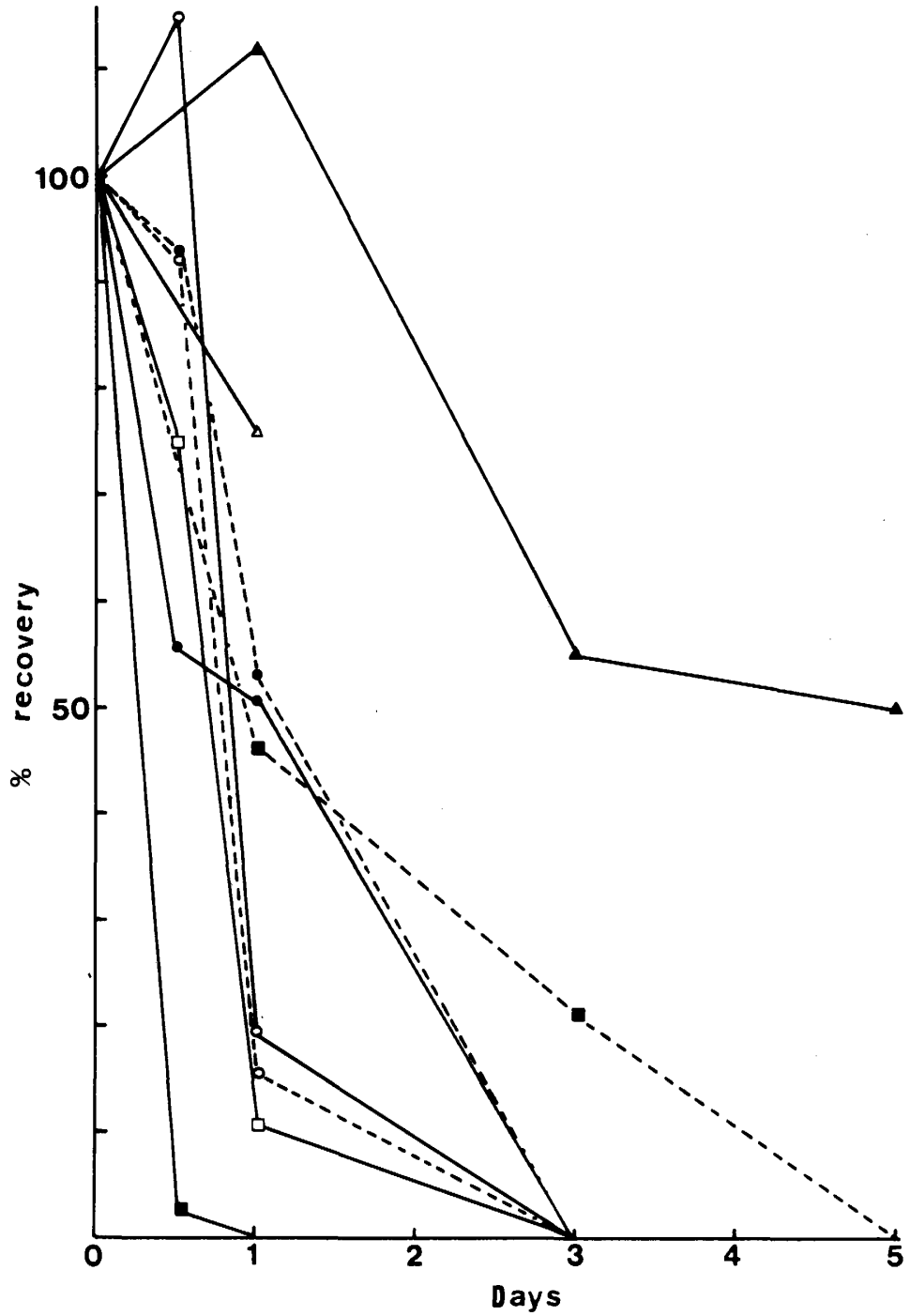
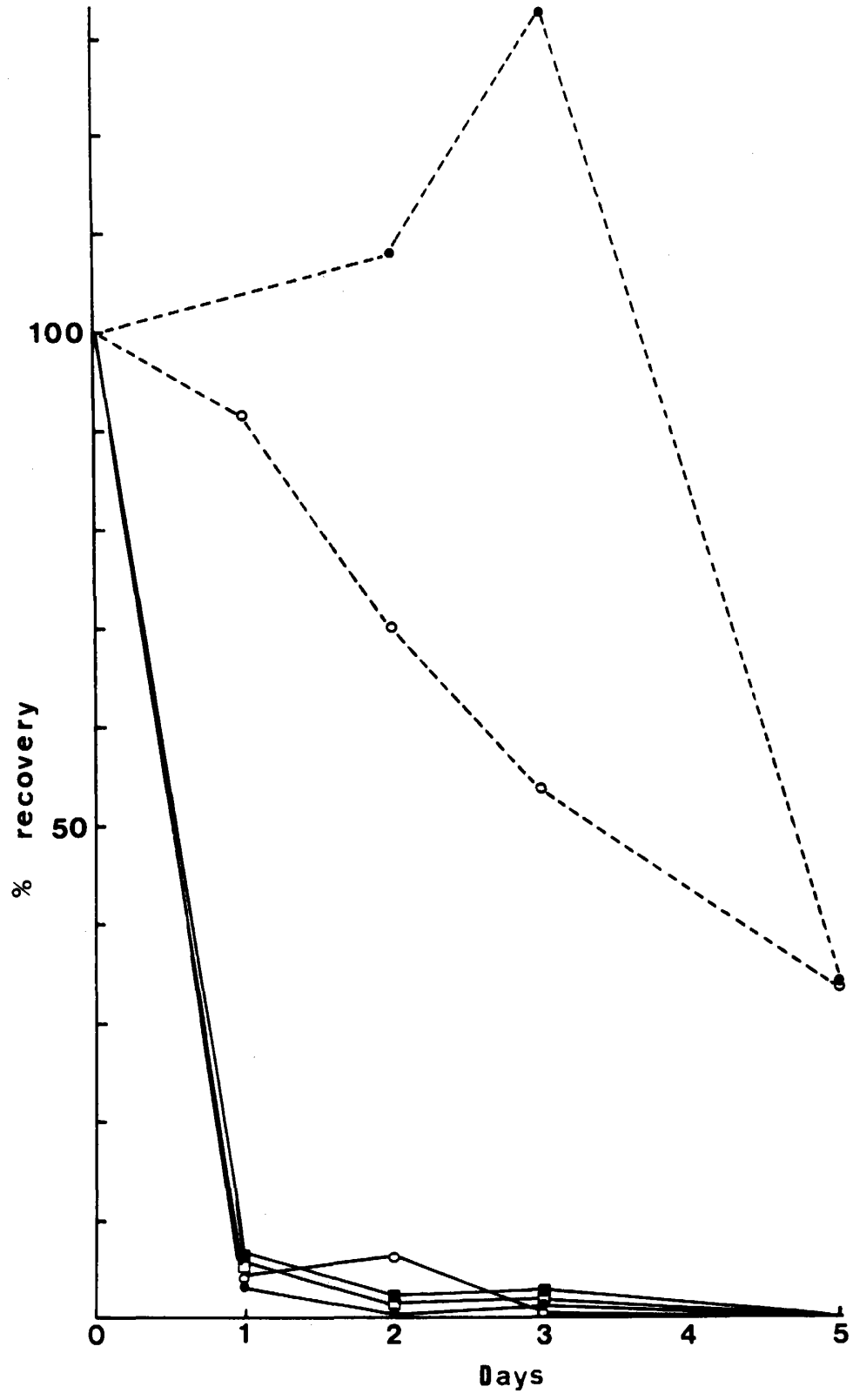


Figure 1 The surviral rate, expressed as % recovery (each point recovery no./initial no. inoculated $\times 100$), of *L. braziliensis*, *L. donovani* and *T. cruzi* in intraperitoneal (IP), subcutaneous (SC) and intrascrotal (IS) chambers in hamsters. Each point shows mean % of 2 to 4 chambers in which 0.1 m*l*/chamber of promatigotes or epimatigotes (3×10^6 to 1×10^7/m*l*) was inoculated. ○——○, *L. braziliensis* in IP chambers; ●——●, *L. braziliensis* in SC; △——△, *L. braziliensis* in IS; □——□, *L. donovani* in IP; ■——■, *L. donovani* in SC; ▲——▲, *L. donovani* in IS; ○----○, *T. cruzi* in IP; ●----●, *T. cruzi* in SC; ■----■, *T. cruzi* in IS.


100
50
% recovery
0
1
2
3
4
5
Days

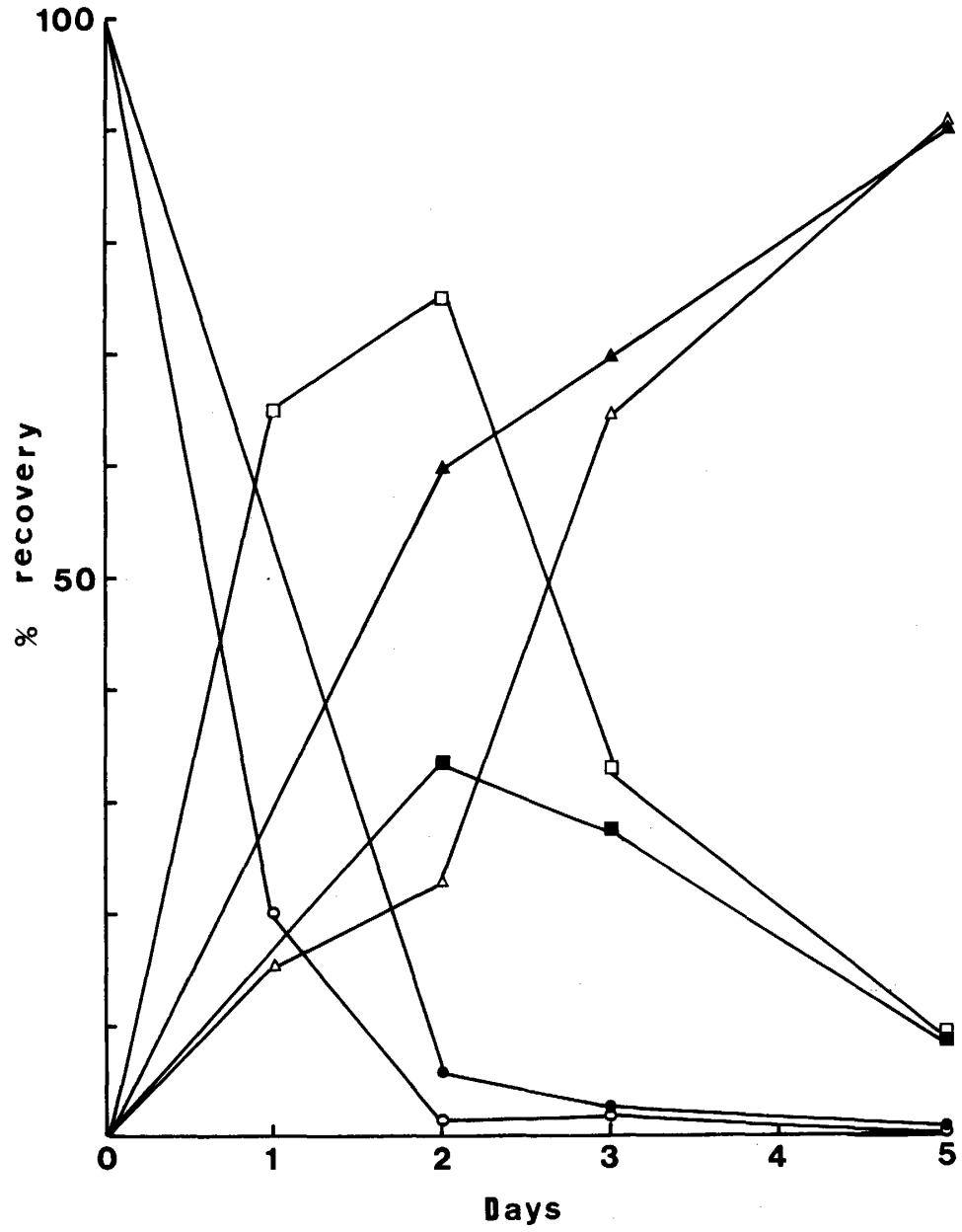


Figure 3 Morphogenetic process of *T. cruzi* in intraperitoneal (IP) and subcutaneous (SC) chambers in mice. Each point indicates the rate, expressed as % recovery, of each stage calculated by counting around 100 protozoans in stained specimens. ○——○, epimastigotes in IP; □——□, trypomastigotes in IP; △——△, amastigotes in IP; ●——●, epimastigotes in SC; ■——■, trypomastigotes in SC; ▲——▲, amastigotes in SC.

Figure 2 The survival rate, expressed as % recovery, of *L. braziliensis*, *L. donovani* and *T. cruzi* in intraperitoneal (IP) and subcutaneous (SC) chambers in mice. Each point shows mean % of 2 to 4 chambers in which 0.1 ml/chamber of promastigotes or epimastigotes (3×10^6 to 1×10^7/ml) was inoculated. ○——○, *L. braziliensis* in IP chambers; ●——●, *L. braziliensis* in SC; □——□, *L. donovani* in IP; ■——■, *L. donovani* in SC; ○----○, *T. cruzi* in IP; ●----●, *T. cruzi* in SC.

A sample of the chamber contents was recovered by insertion of a 27 guage of syringe, and the number of motile parasites was determined by using haemocytometer. An additional sample of the chamber contents was recovered, smeared on a clear microscopic slide, dried, fixed in methanol, and stained in Giemsa's or Wright's staining solution. The morphological stages present in the chambers were studied from these stained specimens with the aid of a compound microscope (1,000×). In some cases, a chamber was examined several times at intervals, by recovering a small quantity of chamber fluid, and then reimplanted into the same host animal.

Results

In hamsters, *L. braziliensis, L. donovani* and *T. cruzi* showed an unexpectedly short survival in intraperitoneal (IP) and subcutaneous (SC) chambers (Figure 1). No marked site preference of the organisms was observed between IP and SC chambers. All the 3 species of parasites, however, tended to demonstrate a longer time of survival in intrascrotal (IS) chambers than in IP and SC ones. In the host animal, no transfomation of the 2 genera, *Leishmania* and *Trypanosoma,* was observed in IP, SC and IS chambers, and the majority of the parasites revealed a small, round and dwarf form with an extremely short flagellum.

In mice, almost all of the leishmanial parasites, *L. braziliensis* and *L. donovani,* have died in IP and SC chambers within 1 day after implantation (Figure 2). *T. cruzi,* on the other hand, survived well in both IP and SC chambers. The organisms in SC chambers multiplied during 2 and 3 days after implantation, but thereafter they gradually decreased and reached 34% of the initial numbers, on day 5. In *L. braziliensis* and *L. donovani,* no transformation of the parasites was observed, while *T. cruzi* transformed into trypomastigote and amastigote in IP and SC chambers, as shown in Figure 3. In IP chambers, transformation of *T. cruzi* from epimastigote to trypomastigote occurred at 65% on the day 1 when 15% of the parasites were recovered as amastigote form. On day 5, around 90% of *T. cruzi* was recognized as amastigote in both IP and SC chambers, but a small rate of the parasites still remained epimastigote or trypomastigote. In the present study no IS chamber was examined in mice, because of too small size of the scrotum to insert the diffusion chamber.

Discussion

The current study was designed to investigate the transformation and survival of *L. braziliensis* and *L. donovani,* together with *T. cruzi,* in IP, SC and IS chambers in hamsters and mice. In the 2 species of the genus *Leishmania,* however, an unexpectedly short survival of the parasites was observed especially in IP and SC chambers in the both host animals, though the parasites in IS chambers revealed a relatively good result. The parasites, furthermore, could not perform transformation and most of them were aberrant forms of the promastigote with an extremely short flagellum. The results obtained indicated that the present condition of chambers implanted was not suitable for *L. braziliensis* and *L. donovani.* But, it was noticeable that the leishmanial parasites tended to survive for a longer time in IS chambers than in IP and SC ones in hamsters. The precise reasons for this better survival were not clear, but it might be due to lower temperature of the scrotum as compared with other body sites, and also due to some immunological and physiological conditions of the organ. It has been well known that the intrascrotal temperature in mammals is considerably below the general body temperature

(Baker, 1986). The intrascrotal region of hamster, therefore, might be a site of choice for the *Leishmania* species in such a study.

T. cruzi, on the other hand, survived well performing morphogenesis in IP and SC chambers in mice, but not in hamsters. As to trasformation of trypanosomes, temperature has been believed as an important factor by several workers. In *T. cruzi,* most of the parasites could be stimulated to transform into trypomastigote, by raising the temperature to 37°C and lowering the pH in axenic culture or tissue culture (Pan, 1971; Trejos *et al.*, 1963). However, there are other facts that *T. cruzi* did not transform in the diffusion chambers either maintained in *in vitro* cell culture at 37°C or in several types of *in vitro* culture media at 26°C or 37°C (Logan and Hanson, 1974). Thus, the influence of temperature might be relatively minor in the transformation of trypanosomes, as compared with other factors (Logan and Hanson, 1974). The morphogenetic process of *T. cruzi* was established when the parasite was maintained in IP chambers in mice (Logan and Hanson, 1974), as same as the present study. Therefore, the factors necessary for the transformation of these protozoans should be further studied in future by using *in vitro* and *in vivo* systems.

From the results obtained, it was concluded that the leishmanial parasites, obligatory intracellular organisms in mammalian hosts, could not survive for a long time and could not perform transformation in diffusion chambers implanted into hamsters or mice. The results also suggested that the factors responsible for the transformation and survival of the organisms might be greatly different between *Leishmania* and *Trypanosoma,* and also between the 2 host animals, hamsters and mice.

Acknowledgements

We thank the late Professor K. Asami, Department of Parasitology, Keio University School of Medicine, Tokyo, Japan for kind supply of *L. braziliensis* and *L. donovani.* Thanks are also due to Drs. N. Suzuki and H. Osaki of Kochi Medical School, Kochi, Japan for their encouragement throughout the study.

References

1) Aljeboori, T. I. (1979): A simple diphasic medium lacking whole blood for culturing *Leishmania* spp, Trans. Roy. Soc. Trop. Med. Hyg., 73, 117
2) Baker, J. R. (1986): Intrascrotal development of trypanosomes, Parasit. Today, 2, 249
3) Ballon-Landa, G., Douglas, H., Colmerauer, M. M., Goddard, D. and Davis, C. E. (1985): Growth and antigenic variation of *Trypanosoma brucei, T. rhodesiense* and *T. gambiense* in subcutaneous millipore chambers, Trans. Roy. Soc. Trop. Med. Hyg., 79, 24–28
4) Logan, L. L. and Hanson, W. (1974): *Trypanosoma cruzi*: Morphogenesis in diffusion chambers in the mouse peritoneal cavity and attempted stimulation of host immunity, Exp. Parasitol., 36, 439–454
5) Pan, A. A. (1984): *Leishmania mexicana*: Serial cultivation of intracellular stages in a cell-free medium, Exp. Parasitol., 58, 72–80
6) Pan, C. T. (1971): Cultivation and morphogenesis of *Trypanosoma cruzi* in improved liquid media, J. Protozool., 18, 556–560
7) Trejos, A., Godoy, G. A., Greenblatt, C. and Cedillos, R. (1963): Effect of temperature on morphologic variation of *Schizotrypanum cruzi* in tissue culture, Exp. Parasitol., 13, 211–218

Diffusion chamber 内 *Leishmania braziliensis, L. donovani* および *Trypanosoma cruzi* のハムスターとマウス体内での運命 ——予報——

橋口　義久[1]・古谷　正人[2]・岡村　宜典[1]

宿主動物と *Leishmania braziliensis* および *L. donovani* との相互関係を *in vivo* で解析する一つの試みとして，上記原虫を diffusion chamber に封入，ハムスターおよびマウスの皮下，腹腔，陰嚢（ハムスターのみ）へ外科的に移植，継時的に chamber を回収し，まず，それらの世代転換（transformation）や生存を調べた。また比較のため，*Trypanosoma cruzi* についても同様な実験を試みた。その結果，2種の *Leishmania* はハムスターおよびマウスの皮下，腹腔内では，極めて短期間の生存を示したが，ハムスターの陰嚢内 chamber ではより長時間生存した（図1，2）。しかし，いずれの場合にも promastigote から amastigote への転換は認められず，原虫は短鞭毛・小形の移行型に止まった。一方，*T. cruzi* はハムスターでは生存が短いものの，マウスでは腹腔と皮下の両移植部位において5日以上にわたって生存し，epimastigote から trypomastigote, amastigote への転換も認められた（図3）。以上の結果は，*Leishmania* 属と *Trypanosoma* 属間での世代転換が極めて異なること，また両属原虫の生存期間は，diffusion chamber 内という特殊な条件下においても，宿主動物の種によって異なることが示唆された。

1　高知医科大学寄生虫学教室　　2 高知医科大学動物実験施設