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VISCERAL LEISHMANIASIS IN THE GOLDEN HAMSTER  
AS A MODEL FOR HUMAN KALA-AZAR

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ANNUAL REPORT

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council (DHHS, PHS, NIH Publication No. 86-23, Revised 1985).

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Visceral Leishmaniasis in the Golden Hamster  
as a Model for Human Kala-azar

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Experimental Infections with Geographical Isolates of Leishmaniasis

During the last year, we have continued to maintain and characterize various Leishmania isolates of both human and animal origin. Almost all of the isolates originated from visceral infections. A number of these parasites are now routinely carried in hamsters including two L. infantum strains from France and Greece, two L. chagasi strains from Brazil, and a L. donovani strain from the Sudan. In a previous progress report (January, 1983), we also reported extensively on infections with a Honduran strain (WR116; Santos Herrera) isolated from the bone marrow of a human with visceral leishmaniasis. This organism was interesting in that we could produce fulminating dermal lesions in hamsters, but not systemic infections. Inoculation of WR116 intradermally into mice produced cutaneous, ulcerating lesions, some of which appeared to heal and then subsequently relapse to produce chronic, ulcerative lesions. C57BL/6 mice infected with WR116 were not protected against L. donovani inoculated IV; however cross-protection was observed between WR116 and a strain of L. mexicana amazonensis. This last observation made questionable, previous characterizations of WR116 as L. chagasi. We have subsequently studied the behavior of WR116 in mouse macrophage cultures and observed that the parasite grows intracellularly in large, vaculated phagolysosomes in a manner similar to L. mexicana. The organism was sent to Dr. Phillip Scott (NIH) to utilize in

intracellular killing assays and was observed to produce two distinct types of infection in macrophages, one of which is characteristic of L. mexicana. It is now our opinion that WR116 contains 2 parasite species, one L. mexicana and the other a possible L. braziliensis strain. Whether this organism is the same one isolated from human bone marrow is now in doubt.

By far the most interesting visceral isolate we have studied this past year is strain WR503 isolated from a dog during a recent outbreak of leishmaniasis in Oklahoma. This parasite is presumably a strain of L. chagasi or L. infantum and produced classical visceral infections in dogs. Our initial attempts to infect hamsters utilized cultured promastigotes inoculated either intracardially or intradermally into the nose or shaved flank. Although no parasites were recovered from cutaneous sites, however we were successful in culturing promastigotes from the spleen of an IC inoculated animal. Approximately 9 passages in hamsters were required before the first microscopically patent visceral infection was observed. Subsequent passages, often using a dose as high as  $1-2 \times 10^8$  promastigotes injected IC, have yielded low level infections. Since our early objective was merely to establish this strain in hamsters, we often sacrificed animals at random time intervals to determine visceral parasite burdens. Analysis of the numerical data from specific groups of animals, however, has turned up an interesting trend in the course of infection. In contrast to all other strains of L. donovani (chagasi or infantum) studied in this laboratory, the WR503 strain appears to spontaneously resolve following IC inoculation into hamsters. The data in Figure I

represent hepatic parasite burdens in 4 groups of hamsters receiving this parasite. Splenic parasite burdens are not graphed, but were found to be low in most animals with peak numbers only about  $2-5 \times 10^4$  parasites/spleen.

In an attempt to boost parasite burdens in WR503 infected hamsters, animals were treated twice weekly with 2.5 mg/100g cortisone acetate during the first 2 weeks of infection. Cortisone treated, as well as non-treated, hamsters were sacrificed at 4 weeks and parasite burdens were determined. In contrast to control animals, most of which did not harbor microscopically patent infections, cortisone-treated animals averaged over  $10^9$  hepatic amastigotes. The results from two separate groups of animals are shown in Figure 2. The dramatic effect of cortisone on the course of infection suggests that a host response is controlling parasite numbers in vivo.

We have also been able to produce low level visceral infections in mice with WR503. Inoculation of  $2 \times 10^7$  promastigotes IV into BALB/c mice led to the establishment of approximately 7% of the inoculum as hepatic amastigotes as compared to approximately 15% for promastigotes of the 2S strain of L. donovani. While the 2S strain routinely increases 50 fold or more during the first 14 days of infection, the WR503 increased by only 1.5 fold (Figure 3). Whether this low increase in parasite numbers represents a low reproductive rate of this strain or is a reflection of parasite susceptibility to a host response has yet to be determined.

One additional experiment has been performed with the WR503 strain. Individual animals from groups of hamsters which had been shown to resolve infection were pooled and challenged IC, along with controls, with approximately 5-10 million amastigotes of the 2S strain of L. donovani. These animals were then sacrificed 1 week later. The control hamsters averaged greater than  $10^8$  hepatic parasites while the "WR503 healed" animals harbored approximately  $3 \times 10^6$  parasites (Figure 4). Thus, it appears that animals resolving infection with this parasite strain will express resistance against a more virulent strain of L. donovani.

Studies with an isolate from a U.S. soldier in Panama (L. chagasi; WR317) were prompted by the fact that this organism came from a cutaneous lesion. A demotropic strain of L. chagasi would have obvious implications in terms of vaccination against visceral leishmaniasis. In our hands, however, this organism behaves exactly like other L. chagasi strains we have studied. To date, we have not observed any evidence of cutaneous lesions in either mice or hamsters follow intradermal inoculation of this parasite. Following intravenous inoculation into BALB/c mice, hepatic parasite burdens increased by 32 fold from 1 to 21 days which is within the range of the Santana strain of L. chagasi. Mice inoculated intradermally with this strain develop no demonstrable resistance to an intravenous challenge infection with L. chagasi (Santana) which is similar to results we have previously obtained showing that mice inoculated intradermally with 2S strain of L. donovani develop little immunity to intravascular challenge infections. In hamsters, however, dermal

parasite inoculation with WR317 does induce resistance to an IC challenge with the Santana strain (Figure 5), but this resistance is no greater than that seen with other visceral isolates.

One additional observation on strain behavior in animals deserves note. As part of an attempt to identify visceral strains of Leishmania which can produce consistent visceral infections in hamsters following ID inoculation, a large group of animals was inoculated with the Santana strain of L. chagasi. Thus far, none of these animals have developed microscopically patent infections. However, we have been able to culture promastigotes from the spleens of these hamsters as long as 7 months following inoculation. The implications of these persistent low level infections are yet to be determined.

#### Immunity to L. donovani in the Golden Hamster

Much of our effort during the past years concentrated on the study of immunological responses in hamsters infected with the 2S (Sudan) strain of L. donovani. Briefly, we have utilized three model systems:

- 1) IC infections - Inoculation of  $1-10 \times 10^6$  amastigotes intracardially results in a progressive visceral disease in which parasites multiply unchecked in spleen and liver tissue, and death ultimately results from a fulminating infection.

- 2) ID infections - Inoculation of  $1-10 \times 10^6$  amastigotes intradermally into hamsters results in transient dermal lesions which usually resolve within 6-8 weeks. These animals display significant acquired resistance to re-infection.
- 3) ID - IC Challenge infections - Inoculation of  $1-10^6$  amastigotes ID followed several weeks later by an IC challenge with similar numbers of organisms results in visceral infections in which splenic and hepatic parasite burdens are significantly lower than those seen in primary IC infections. Acquired resistance is not absolute, however, since parasite numbers eventually increase and animals ultimately succumb to infection.

To briefly reiterate our previous results, we have found that spleen cells from IC inoculated animals show a depressed ability to respond to Con A as the infection progresses. Cells from hamsters infected for 6-8 weeks are almost totally nonresponsive to Con A and these depressed responses are not augmented by indomethacin. We also compared Con A responses by spleen cells from IC vs ID infected hamsters. Only the IC spleen cells show depressed responses to this mitogen; ID spleen cell responses are similar to controls. The most interesting results from this series of experiments came from a comparison of proliferative responses in ID, IC, and ID-IC challenged hamsters.

As previously described, Con A responses by spleen cells from ID infections are normal, whereas those from IC infections are depressed. Of interest was the observation that lymph node cells from the IC infected group remained responsive to Con A, which suggests that depression is limited to heavily parasitized tissues such as the spleen. It also appears that a relative state of resistance, as is seen in the ID-IC challenged group, does not prevent nonspecific immunodepression in the spleen. This is, perhaps, not surprising, since splenic hyperplasia in this group is similar to that in animals receiving only an IC infection.

However, when antigen-specific responses were assessed, the results indicated that an antigen-specific suppressor cell may be operating in these systemically infected animals. Lymph node cells from ID-IC challenged animals did not respond to antigen, even though they did respond to Con A. Since the prior ID infection would provoke a strong, antigen-specific proliferative response, we must assume that the IC challenge induces active suppression of this response. Since non-specific suppression appears to be limited to the spleen, it is likely that parasite-specific suppression is due to a circulating suppressor cell rather than to suppressor macrophages which develop and reside in parasitized spleen.

During the past 6 months, we made a concentrated effort to demonstrate a cell population in IC inoculated hamsters which would suppress antigen-specific proliferative responses. Several experiments were run comparing responses in ID, IC, and ID-IC inoculated hamsters and the results consistently showed that cells

from hamsters with heavy visceral infections responded poorly to parasite antigens. However, when cells from IC infected hamsters were co-cultured with antigen-reactive cells from ID inoculated animals, we often observed unusually high background levels of thymidine uptake characteristic of mixed lymphocyte responses. We thought it possible that the hamsters used in this study (LHC) were not inbred, but extensive testing of cells from normal LHC hamsters gave no indications of MLC reactions. Although we are still not sure of the immunological basis for these unusual responses, we were able to alleviate our problems by utilizing inbred LHC hamsters, rather than outbred animals, as donors for hamster splenic amastigotes. It is possible that spleen tissue contaminants from outbred donors were sensitizing some recipients to hamsters alloantigens and that animals differing at minor loci were responding to these alloantigens. In any case, the resolution of this problem has led to a demonstration of suppressor cells in IC inoculated animals.

Briefly, hamsters inoculated ID served as a source of antigen reactive cells. Animals inoculated IC with either  $1 \times 10^6$  or  $5 \times 10^6$  parasites served as a source of putative suppressor cells. The results, as seen in Table 1, show that IC inoculated animals given  $1 \times 10^6$  amastigotes had antigen-reactive spleen cells and no evidence of suppressor cells at 4 weeks of infection. In contrast, cells from hamsters given  $5 \times 10^6$  amastigotes had depressed antigen-specific spleen cell responses and these spleen cells suppressed responses by cells from ID inoculated animals (Table 2). Since the mean splenic parasite burdens of the two groups of IC inoculated hamsters were

9 x 10<sup>6</sup> and 49 x 10<sup>6</sup> parasites, respectively, we suspect that the development of suppressor cells may be related to the density, as well as the duration, of visceral infection.

FIGURE 1

Courses of Infection of WR503 in Hamsters following IC Inoculation of Promastigotes

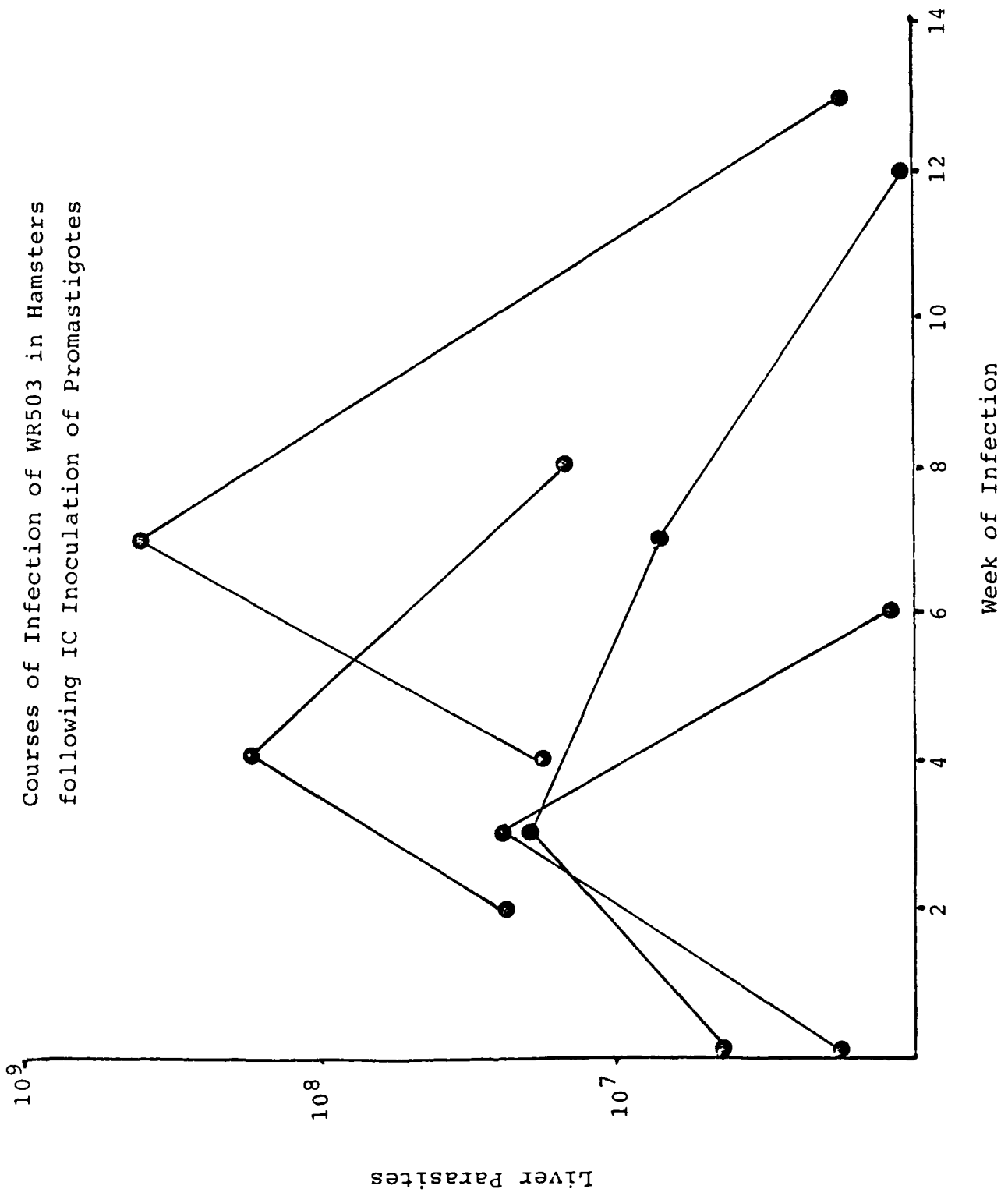


FIGURE 2  
 Effect of Cortisone (2.5 mg/100g) Treatment  
 on Parasite Burdens in Hamsters  
 Infected with WR503

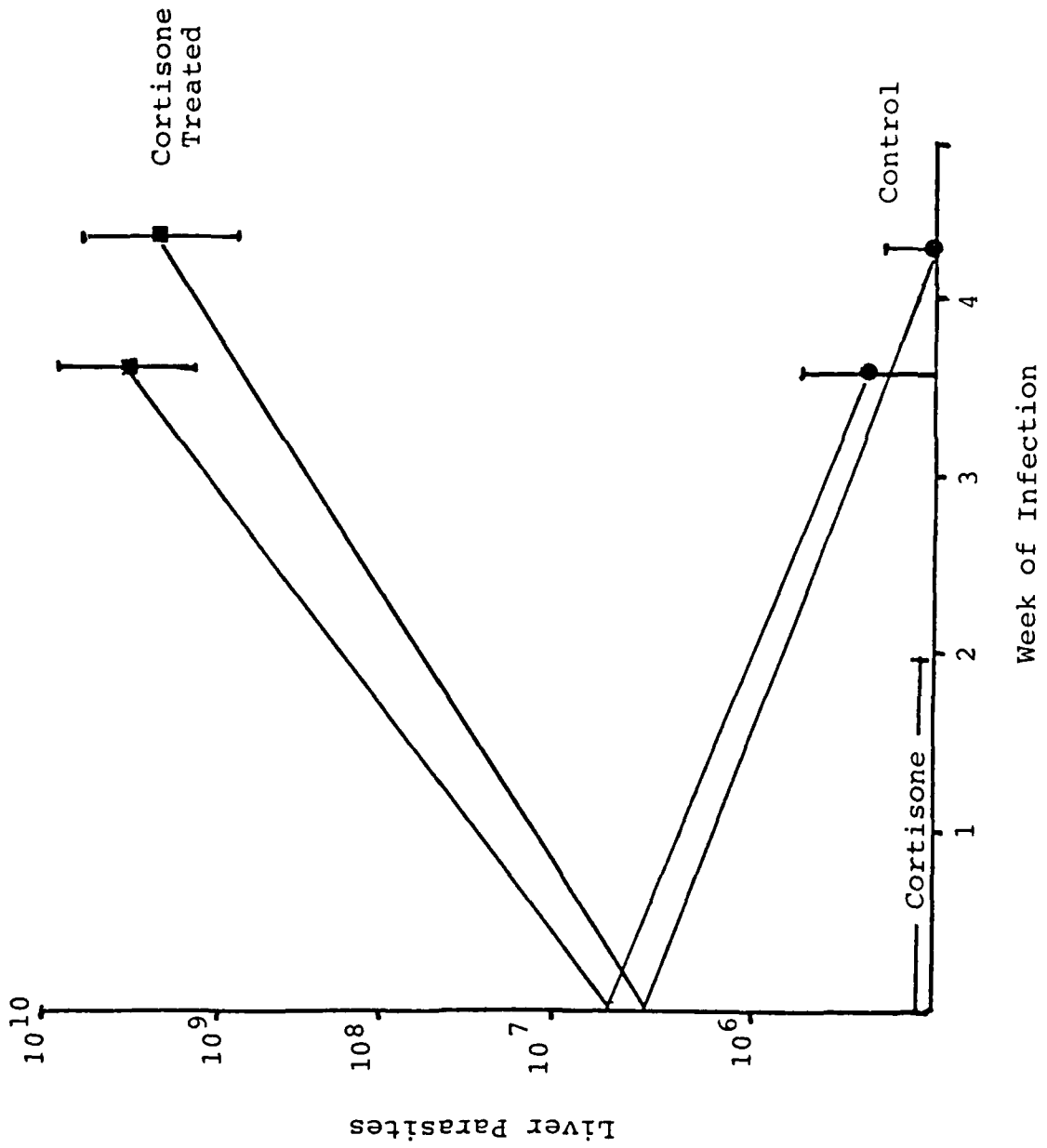


FIGURE 3  
Challenge Infection with L. donovani (2S)  
in Control versus WR503 "Healed" Hamsters

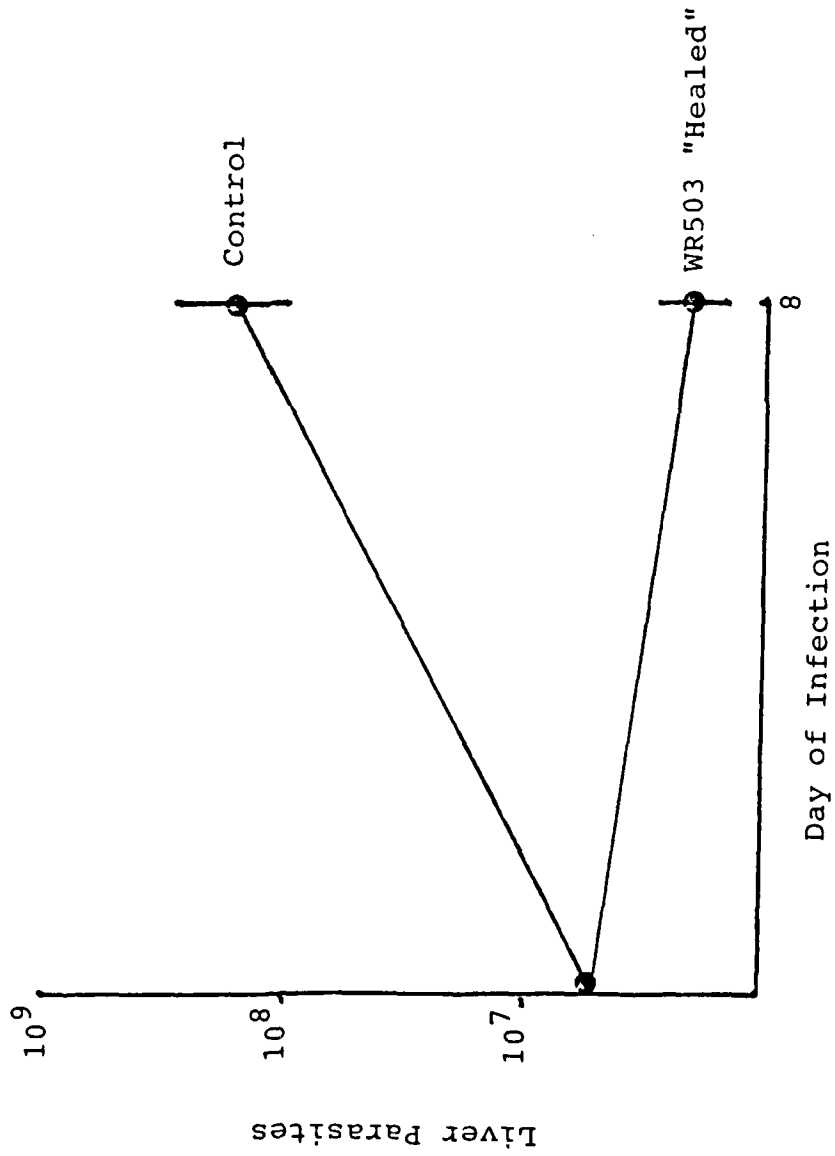


FIGURE 4

Course of Infection of L. donovani (2S) versus WR503 in BALB/c Mice

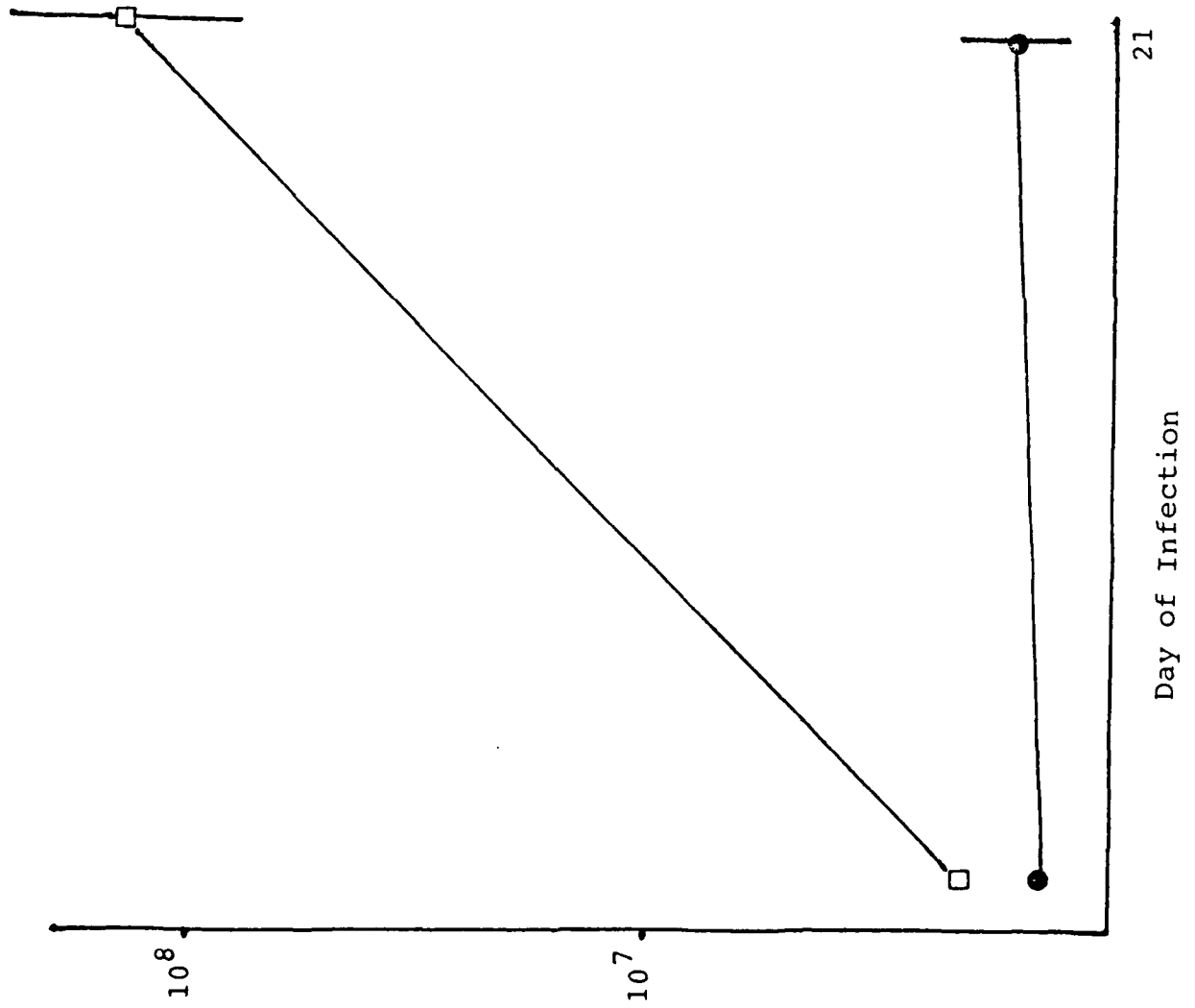


FIGURE 5

L. chagasi (Santana) Challenge Infections  
in Control Hamsters versus Hamsters  
Inoculated Intradermally with WR317

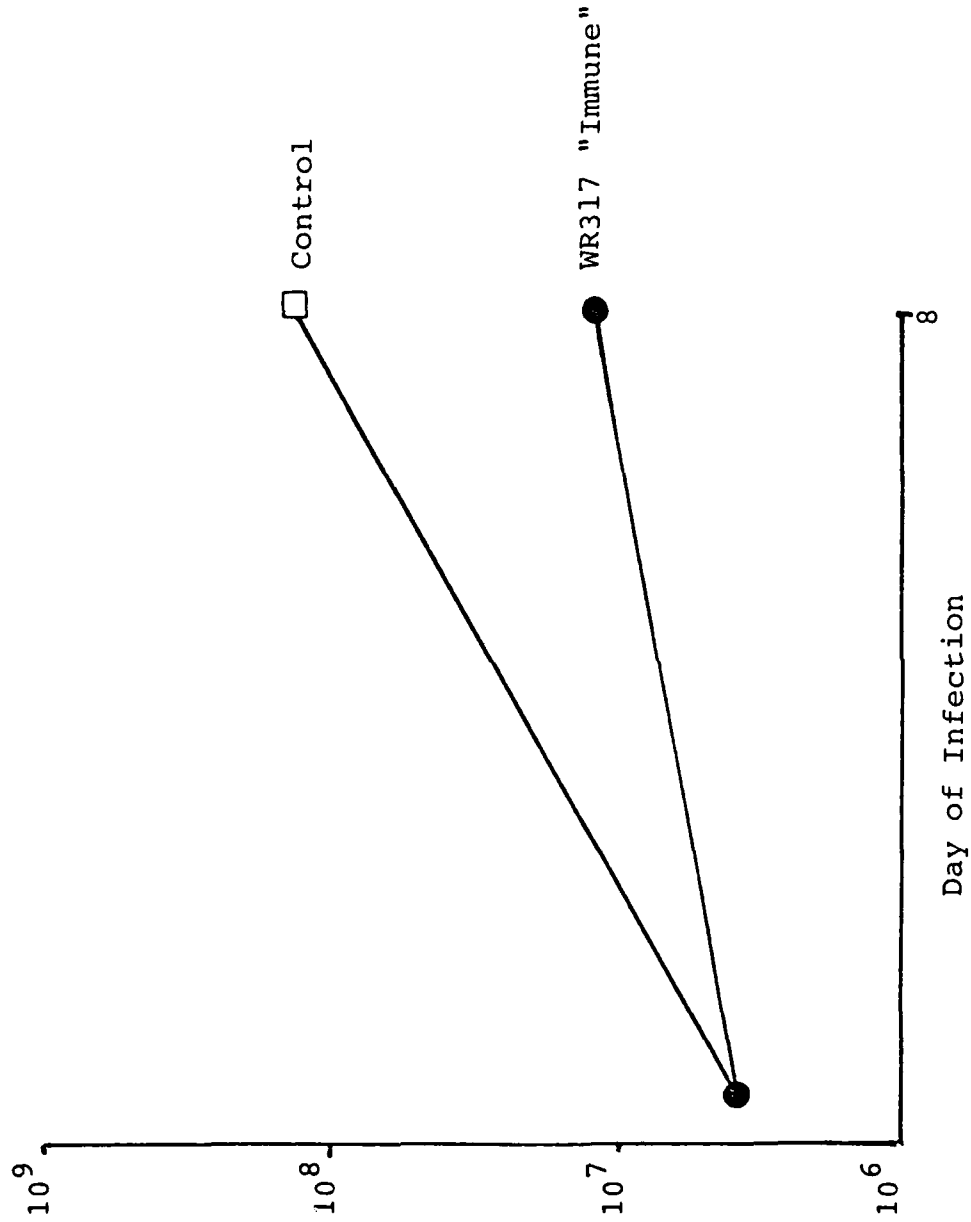


TABLE 1

Antigen-specific Proliferative Responses  
of Spleen Cells from Hamsters Inoculated ID ( $5 \times 10^6$ )  
or IC\* ( $1 \times 10^6$ ) with L. donovani Amastigotes

<u>Cell Type</u>	<u>Thymidine Uptake (CPM)</u>	
	<u>Without Antigen</u>	<u>With Antigen</u>
Normal Spleen	1389	1481
ID Spleen	1825	19,093
IC Spleen	3319	15,997
ID + IC Spleen	2321	17,934
ID + Normal	1600	13,420
IC + Normal	2419	12,615

\* Mean Parasite Burdens at 4 Weeks

Spleen -  $9 \times 10^6$

Liver -  $7.4 \times 10^7$

TABLE 2

Antigen-specific Proliferative Responses  
of Spleen Cells from Hamsters Inoculated ID ( $5 \times 10^6$ )  
or IC\* ( $5 \times 10^6$ ) with L. donovani Amastigotes

<u>Cell Type</u>	<u>Thymidine Uptake (CPM)</u>	
	<u>Without Antigen</u>	<u>With Antigen</u>
Normal Spleen	1147	1850
ID Spleen	2622	17,240
IC Spleen	898	1560
ID + IC Spleen	835	1893
IC + Normal	610	646
ID + Normal	1220	5904

\* Mean Parasite Burdens at 4 Weeks  
Spleen -  $4.9 \times 10^7$   
Liver -  $1.4 \times 10^9$