Interactions between malaria (Plasmodium yoelii) and leishmaniasis (Leishmania mexicana amazonensis), with a note on the blood-feeding behavior of Lutzomyia longipalpis.

INTERACTIONS BETWEEN MALARIA (*PLASMODIUM YOELII*) AND LEISHMANIASIS (*LEISHMANIA MEXICANA AMAZONENSIS*), WITH A NOTE ON THE BLOOD-FEEDING BEHAVIOR OF *LUTZOMYIA LONGIPALPIS*.

A Dissertation Presented

By

Russell Edward Coleman

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To Susan and Derek:

Their love makes it all worthwhile.

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ABSTRACT

INTERACTIONS BETWEEN MALARIA (*PLASMODIUM YOELII*) AND LEISHMANIASIS (*LEISHMANIA MEXICANA AMAZONENSIS*), WITH A NOTE ON THE BLOOD-FEEDING BEHAVIOR OF *LUTZOMYIA LONGIPALPIS*.

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Evidence has suggested that delicate immunological balances that normally prevent establishment of *Leishmania* infections are upset following stress (i.e., malaria), resulting in the development of clinical leishmaniasis. This study was undertaken to determine 1) whether concomitant infection with *Plasmodium yoelii* would result in more severe *Leishmania mexicana* infection 2) whether immunomodulating agents would affect the development of these parasites, 3) whether chemotherapy directed at *L. mexicana* would affect the progression of *P. yoelii*, and 4) whether host anti-vector defensive behavior would be affected during concomitant infections.

Laboratory mice were infected with *L. mexicana* and *P. yoelii*. These mice developed more severe infections than did animals infected with either parasite alone. Maximum disease enhancement resulted when the interval between the inoculation of the two parasites was minimized. Several C/57 mice infected with both pathogens developed

disseminated L. mexicana infections. Dissemination was never observed in control mice.

Leishmania mexicana infected mice were treated with immunomodulating agents. Cimetidine, ranitidine, and 2'-deoxyguanosine were as effective as pentostam, an antileishmanial agent, at limiting parasite development. Plasmodium yoelii infected mice which were treated with pentostam developed more severe malaria than did cimetidine treated mice or control animals. Both agents limited L. mexicana development in mice infected with both L. mexicana and P. yoelii; however, cimetidine reduced P. yoelii infection in these mice whereas pentostam increased the severity of the malaria.

Mosquitoes (Aedes aegypti) were unable to feed on uninfected mice or on mice infected with either P. yoelii or L. mexicana. Significantly more mosquitoes engorged on mice infected with both pathogens. Maximum engorgement occurred during the crisis phase of the enhanced malarial infection. Other parameters which were examined were altered during peak malarial infection in these dually infected animals.

Results of this study indicate that concomitant infection with Plasmodium yoelii and Leishmania mexicana can influence parasite development in laboratory mice. Not only were clinical symptoms affected, but vector feeding success was enhanced during dual infections. These factors may be critical to the epidemiology of the disease. The efficacy of certain chemotherapeutic agents was also markedly affected in these dually infected animals.

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Chapter 1

LITERATURE REVIEW

Malaria

Malaria is caused by protozoan parasites belonging to the genus Plasmodium (Family Plasmodiidae). These organisms undergo two stages of asexual reproduction (schizogony) in the vertebrate host and one stage of sexual reproduction (sporogony) in the mosquito vector. Asexual reproduction initially occurs in the parenchymal cells of the liver, followed by a subsequent erythrocytic cycle (Bruce-Chwatt 1985).

There are approximately 120 recognized Plasmodium; of these, only P. falciparum, P. vivax, P. ovale, and P. malariae infect humans (Garnham 1966 1980). The remaining species are parasites of birds, reptiles, primates, rodents, bats, and various other small mammals (Bruce-Chwatt 1985, Killick-Kendrick & Peters 1978). Sixty species of anopheline mosquitoes (Diptera: Culicidae) transmit P. falciparum, P. vivax, P. ovale, and P. malariae to humans (Bruce-Chwatt 1970, Garnham 1966).

Clinical symptoms of malaria typically include fever and anemia (Aikawa et al. 1974, Maegraith 1977, 1981). Anemia results primarily from the destruction of parasitized erythrocytes, although decreased

production of red blood cells by the bone marrow contributes to the decreased hematocrit (Pasvol & Wilson 1982, Seed & Kreier 1980). Fever presumably results from the release of endogenous pyrogen from leucocytes in response to the production of toxic products of the parasite. The pyrogen interacts with receptors on the hypothalamus, resulting in the release of prostaglandin and monoamines. These agents signal sympathetic nerve fibers to constrict peripheral blood vessels (Bruce-Chwatt 1985, Evered & Whelan 1983).

Leishmaniasis

Leishmaniasis is a vector-borne disease that occurs on all continents except Australia and Antarctica (Zuckerman & Lainson 1977). Leishmaniasis is transmitted by various phlebotomine sand flies (Diptera: Psychodidae) in the genera Phlebotomus and Lutzomyia (Lewis 1974, Ward 1977 1985). Estimates on the annual incidence of the disease range from 400,000 (Chance 1981) to 12 million (Walsh & Warren 1979) human cases. The disease is primarily a zoonosis, with the exception of visceral leishmaniasis in India and on the east coast of China (Chang et al. 1985).

Leishmaniasis is caused by parasites belonging to the protozoan genus Leishmania Ross 1903 (Family: Trypanosomatidae) (Chang et al. 1985). The Leishmania are taxonomically separated into 2 distinct groups, the Peripylaria and the Subpylaria, which are differentiated according to the position of the parasite within the vector (Lainson & Shaw 1979). Six species of Leishmania infect humans: Leishmania

mexicana, L. major, L. donovoni, L. braziliensis, L. tropica, and L. aethiopica (Molyneux & Ashford 1983). The parasite exists as an extracellular flagellated form in the digestive tract of phlebotomine sand flies and as a non-motile amastigote in the macrophage of the vertebrate host (Molyneux & Ashford 1983). Amastigotes are taken up by the sand fly during feeding. The amastigotes differentiate into promastigotes and begin to divide soon after ingestion. The parasites initially colonize the midgut or hindgut of the fly (Killick-Kendrick et al. 1974, Rioux et al. 1979). Colonization subsequently extends forward until the pharynx is reached. There, promastigotes attach to the cuticle (Killick-Kendrick 1979, Killick-Kendrick et al. 1974, Walters et al. 1987). Promastigotes can eventually be found in the proboscis of the fly, from where they find their way into the skin of the mammalian host during feeding (Chang et al. 1985). Promastigotes are initially non-infective to the vertebrate; however, sequential development leads to the production of infective forms (Sacks & Perkins 1984, 1985).

Numerous studies have shown that infected sand flies have difficulty in obtaining blood (Adler & Ber 1941, Chung et al. 1951, Strangways-Dixon & Lainson 1966, Williams 1966). Killick-Kendrick et al. (1977) observed that infected sand flies took second and third blood meals with great difficulty, and Killick-Kendrick and Molyneux (1981) suggested that promastigotes interfered with sensilla controlling probing and engorgement. Sensilla in the proboscis have been described by Killick-Kendrick and Molyneux (1981), and Lewis (1984) described the cibarial sensilla of sand flies. Interference with the

sensilla could result in parasite detachment from the pharynx and lead to their deposition in the skin of the host. Recent studies have clearly shown that vector probing ability is altered when parasites are present (Beach et al. 1984, 1985, Killick-Kendrick et al. 1985). Congestion of parasites at the stomodeal valve and in the anterior foregut may result in a backwash of blood during feeding, resulting in the egestion of parasites (Jefferies et al. 1986, Warburg & Schlein 1986).

Once in the host, invading promastigotes are rapidly taken up by macrophages, where transformation to the amastigote form occurs. Parasites rapidly divide and the macrophage eventually ruptures, releasing the parasites into the tissue from where they invade fresh cells (Molyneux & Ashford 1983).

Three distinct forms of leishmaniasis occur in humans. These are visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis (Marsden & Jones 1985). Visceral leishmaniasis is caused by L. donovoni donovoni (Naik 1979), L. donovoni infantum (Bettini et al. 1981) or L. donovoni chagasi. Following inoculation of promastigotes by the sand fly vector, the parasites are taken up by macrophages in the liver, spleen, bone marrow, and lymph nodes. The characteristic clinical symptom at this stage of infection is an intermittent fever. The disease rapidly progresses unless appropriate therapy is initiated. Symptoms during advanced infection include prolonged fever and hepatosplenomegaly. Death normally results from bacterial sepsis or hemorrhage (Marsden & Jones 1985).

Cutaneous leishmaniasis is caused by several species of Leishmania, including L. mexicana, L. braziliensis, L. tropica, and L. major (Lainson & Shaw 1973, Manson-Bahr 1971). As with other forms of leishmaniasis, promastigotes are inoculated into the skin by sand flies. Amastigotes are taken up by macrophages, undergo division, rupture the cell, and are taken up by fresh macrophages. The host mounts an immune response in which an initial invasion of polymorphonuclear leukocytes is followed by an infiltration of lymphocytes. Ulceration results from a combination of cellular infiltration, edema, and vasculitis, leading to a poor blood supply and necrosis (Marsden & Jones 1985). Blood stream dissemination of parasites occurs rarely.

Leishmania braziliensis braziliensis is the primary pathogen responsible for mucocutaneous leishmaniasis. Parasites metastasize from an original skin lesion to the blood vessels around the nasal septum. Parasites may then spread to the buccal cavity, pharynx, and larynx (Molyneux & Ashford 1983).

Parasite Interactions

Despite extensive control efforts, vector-borne diseases remain an immense obstacle to social and economic development in the tropics (BOSTID 1983). Malaria, filariasis (including onchoceriasis), trypanosomiasis, and leishmaniasis debilitate hundreds of millions of people annually and have resisted most of our control efforts. The resurgence of malaria is of particular concern, and malaria is now

considered endemic or epidemic throughout much of Africa, southern Asia, and South and Central America. The World Health Organization (WHO) initiated its campaign to eradicate malaria in 1956, and by 1970, 727 million people were no longer at risk from malaria (WHO 1979). As recently as 1978, popular literature had reported that malaria had been wiped out (Phillips 1983); however, nothing could be further from the truth. In Africa, where the WHO eradication program was never implemented, malaria control efforts have had little success, and malaria remains an indigenous problem to the general population. In other countries, initial success has been replaced with a dramatic resurgence of malaria (Bruce-Chwatt 1985). Drug resistant Plasmodium, insecticide resistant Anopheles mosquitoes, poor anti-mosquito control measures, as well as financial and administrative shortcomings have all led to this rebound (WHO 1979). Sri Lanka, India, Pakistan, Bangladesh, and Turkey are notable for the rising incidence of malaria following the almost total elimination of the disease in these countries (WHO 1983).

Resurgence of malaria in these third-world countries has led to an increased number of problems which can be directly attributed to malaria outbreaks, such as increased medical costs, depletion of limited fiscal resources, and loss of labor. Lower infant birth rates and higher rates of prenatal mortality have been reported (Brabin 1983, Bray & Anderson 1979, Bruce-Chwatt 1983, Gilles et al. 1969, Jelliffe 1968), while the incidence of lymphoma's has increased greatly in malaria endemic areas (Salaman 1970). Indirect effects of malaria, such as interaction with concomitant pathogens, have been

reported. Historically, suppression of Treponema pallidum, a causative organism of neurosyphilis, via malarial febrile paroxyms, was a noted medical benefit of pathogen interaction (Bruce-Chwatt 1985).

Enhancement, as well as suppression, of concomitant infections has also been attributed to Plasmodium infection. Many infectious diseases, such as leishmaniasis, trypanosomiasis, and shistosomiasis, coincide geographically with malaria. Malaria may influence the course of these diseases, changing an otherwise non-lethal infection into one with serious sequelae. Children infected with P. falciparum are abnormally susceptible to bacterial infections (Greenwood et al. 1971), and malaria has been shown to exert an effect on murine oncogenic viruses (Salaman et al. 1969), granuloma formation by Shistosoma mansoni (Abdel-Wahab et al. 1974, Lwin et al. 1982), and on the spontaneous invasion of tissue by Giardia muris (Radelescu et al. 1971). Numerous other studies have corroborated the enhancing role Plasmodium infections can have on concurrent microbial infections (Bomford & Wedderburn 1973, Thurston 1955, Strickland et al. 1972).

The immunosuppressive role of malaria appears to be almost entirely responsible for these enhanced infections (Killick-Kendrick & Peters 1978). Immunosuppressive effects of malaria have been documented in a number of laboratory studies (Freeman 1978, Greenwood et al. 1971, Khansari et al. 1981, Lelchuk & Playfair 1980); however, the mechanism has not been entirely elucidated (Khansari et al. 1981). Suppression of humoral responses is clear (Barker 1971); the role of cell-mediated immunosuppression less so (Zuckerman 1977).

The rodent malaria, P. berghei, purportedly exerts its immunosuppressive effect by an action either on bone-marrow derived lymphocytes or on macrophages (Greenwood et al. 1971). More recently, suppressor cells derived from T and macrophage lineages have been implicated (Weinbaum et al. 1978, Correa et al. 1980), as have soluble secreted substances (Khansari et al. 1981). Functional impairment of relevant immunological cells may also result in immunosuppression (Greenwood et al. 1971, Loose et al. 1972, Wedderburn 1974). Additional research is needed before the precise mechanism of Plasmodium mediated immunosuppression is determined.

Leishmaniasis coincides geographically with human malaria in numerous areas of the world (WHO 1984, Harwood & James 1979). Evidence suggests that species of Leishmania, as well as Plasmodium species, exert strong immunosuppressive effects (WHO 1984). Suppression of cellular immunity mediated by macrophages and sensitized T cells appears to be the primary mechanism of Leishmania mediated immunosuppression (Howard et al. 1980, Nickol & Bonventre 1985a 1985b 1985c). Unlike malaria, leishmaniasis is primarily and endemic disease with normally localized occurrence. In humans, many Leishmania infections terminate subclinically (Molyneux & Ashford 1983, WHO 1984). Studies have indicated that exposure to L. donovoni does not necessarily result in clinical symptoms (Fuller et al. 1979, Pampiglione et al. 1974). Up to 64% of individuals examined in Ethiopia tested positive for the Leishmanin skin test. However, only 2 of the 2723 individuals tested had active L. donovoni infections (Fuller et al. 1979). This suggests a high incidence of cryptic

infections (Fuller et al. 1979). Circumstantial evidence suggests that delicate immunological balances that normally prevent establishment of Leishmania infections are commonly upset following stress (e.g., famine, war, and epidemic malaria) and result in the development of clinical leishmaniasis (Corkill 1948).

The potential for both concurrent and asynchronous Plasmodium - Leishmania infections readily exists, based on their respective geographic ranges and seasonal patterns. Geographic overlap, in conjunction with the known immunosuppressive effects of each parasite, suggests that concurrent Plasmodium and Leishmania infections could each suppress immune function. This would theoretically increase the severity of the complementary disease. Decreased immunocompetence following recovery from acute P. falciparum infection has been demonstrated (Greenwood et al. 1972, McGregor 1972). Hence, dual infections need not be simultaneous in order for disease enhancement of occur.

The incidence of leishmaniasis has been shown to decrease markedly in areas where intensive malaria control efforts are implemented (Molyneux & Ashford 1983). Elimination of endophilic sand flies due to the use of residual pesticides directed at Anopheles mosquitoes has been suggested as the primary cause of this decrease. However, epidemiological evidence suggests that leishmaniasis is predominantly a rural disease. High risk groups include military personnel, woodcutters, hunters, etc., indicating that endophilic sand flies are probably not the primary vectors of leishmaniasis in many instances. We suggest that it may be the absence of Plasmodium

infections (resulting from malarial control efforts), rather than the elimination of suitable vectors, that resulted in the corresponding decrease in the incidence of clinical cases of leishmaniasis.

In the only experimental study of dual malaria - leishmaniasis infections, the interactions between P. berghei and L. infantum was examined in the golden hamster (Adler 1954). Protection against the normally lethal P. berghei was found in hamsters previously infected with a virulent strain of L. infantum. Infection with P. berghei did not inhibit or promote multiplication of L. infantum. The model used by Adler is not directly applicable to typical human infections, as both L. infantum and P. berghei are more highly virulent in the hamster than any human Plasmodium of Leishmania species.

Chemotherapy

Malaria

Different species of Plasmodium vary in their susceptibility to antimalarial drugs, and not all life stages are affected by a given agent (Rieckmann & Silverman 1977). Drugs used for the treatment of malaria can be divided into two major groups of compounds, i) prophylactic agents, which prevent the establishment of the parasite in the liver, and ii) schizonticides, which attack the parasites in the red blood cell (Bruce-Chwatt 1985). Compounds which are effective antimalarial agents include antifolates, 8-aminoquinolines, 4-aminoquinolines, artemisinine, and various antibiotics (WHO 1984b).

Chinchona alkaloids, and in particular quinine, have been used to treat malaria for over 300 years (Bruce-Chwatt 1985). Various new agents which have been synthesized in recent years have largely replaced quinine; however, as the incidence of drug resistant Plasmodium increases, use of quinine is increasing. Quinine is primarily used in treating severe cases of malaria that do not respond to other agents. Prolonged administration of quinine can result in significant side-effects (Rieckmann & Silverman 1977).

The 8-aminoquinolines are active against primary tissue stages, hypnozoites (those liver forms that result in parasite relapse), gametocytes, and asexual blood stages (WHO 1984b). Primaquine, the most widely used 8-aminoquinoline, is not used alone for the treatment or prevention of malaria. This agent is primarily used i) to prevent relapses of malaria, or ii) in conjunction with other agents to prevent the spread of drug resistant parasites (Bruce-Chwatt 1985).

Chloroquine, a 4-aminoquinoline, is the most widely used antimalarial drug. Its initial success was an important component of the WHO global malaria eradication campaign, and the spread of resistance to the compound contributed to the demise of the program (Moore & Lanier 1961, Harinasuta et al. 1962). Chloroquine is effective both as a blood schizontocide and as a prophylactic agent and also destroys gametocytes of P. vivax, P. ovale, and P. malariae, but not P. falciparum. Side-effects are uncommon. The schizontocidal toxicity of chloroquine results from the selective uptake of the agent by infected erythrocytes (WHO 1984b). Chloroquine resistance may result

from the active efflux of chloroquine from infected erythrocytes, effectively preventing the accumulation of toxic levels of the compound in the cell (Martin et al. 1987). Use of verapamil, a calcium channel blocker, can effectively reverse drug resistance by inhibiting the efflux of chloroquine from infected erythrocytes (Martin et al. 1987).

Mefloquine is a substituted 4-quinoline methanol that is structurally similar to quinine. It is a highly efficient schizonticidal that is effective against chloroquine, pyrimethamine resistant P. falciparum and against P. vivax (Bruce-Chwatt 1985). Side effects are minimal at therapeutic doses.

The antifolates include pyrimethamine and proguanil (WHO 1984b). These agents inhibit dihydrofolate reductase or dihydropteroate synthetase and are effective against all multiplying stages of the parasite. Various antibiotics, including monocycline, doxycycline, lincomycin, and clindamycin affect developing liver stages and blood stages. These agents are inhibitors of ribosomal protein synthesis in prokaryotes, and are normally used following treatment with quinine (WHO 1984b). Artemisinine (qinghaosu) is a sesquiterpene lactone isolated from the plant Artemisia annua. Quinghaosu is active against chloroquine resistant P. falciparum in humans, and acts synergistically with mefloquine (WHO 1984b).

Leishmaniasis

All forms of old world cutaneous leishmaniasis heal spontaneously, and in the new world, infection with L. mexicana normally results in benign lesions. Treatment of these forms of disease may not be required unless multiple lesions occur or unless lesions are located on a cosmetically important site (Marsden & Jones 1985). Due to the danger of metastasis and the development of mucocutaneous leishmaniasis, infection with L. braziliensis requires mandatory treatment. Likewise, clinical visceral leishmaniasis can be uniformally fatal unless treatment is initiated (Rees et al. 1985).

All forms of leishmaniasis are initially treated with pentavalent antimony in the form of sodium stibogluconate (pentostam) or N-methyl glucanime antimonate (glucantime) (Berman 1985). The mode of action of these agents is unknown. Treatment failure or relapse occurs in approximately 10-25% of the cases where these agents are used (Berman 1983). Therapeutic doses of pentavalent antimonials are also associated with a variety of toxic side effects. Malaise, anorexia, and vomiting appear early in the treatment, with electrocardiograph alterations and occasional renal insufficiency (Marsden & Jones 1985).

Secondary treatment for leishmaniasis normally consists of pentamidine of amphotericin B. However, these agents are expensive, relatively toxic, and treatments are lengthy (Berman 1985). Intense efforts are therefore currently underway to develop new therapeutic drugs. Agents which show promise include allopurinol (Kager et al. 1981, Walton et al. 1983), nifurtimox (Marsden et al. 1979),

pyrimethamine (Neal 1976), various 8-aminoquinolines (Kinnamon et al. 1978), and chlorpromazine (Pearson et al. 1982).

The use of liposome-encapsulated agents has greatly reduced the amount of drug required to treat leishmaniasis (Alving & Swartz 1984, Reed et al. 1984). Liposomes are vesicles which form spontaneously when lipids are dispersed in aqueous media. Any drugs present in the media will be partially incorporated into the the aqueous phase between the lipid rings. Liposomes are rapidly removed from the circulatory system by the reticuloendethelial cells of the liver and spleen (Juliano 1982, Scherphof 1982). Therapeutic agents incorporated within the liposomes are then available to kill any parasites within the macrophage, but are themselves effectively sequestered from the rest of the body (Black et al. 1977, New et al. 1978, Reed et al. 1984).

Immuno-modulation

The use of therapeutic agents which modulate immune responses could be of great value in the prophylaxis and treatment of infectious diseases (Drews 1985). Three classes of compounds which affect immune function have received widespread attention. These include substances which affect purine metabolism in lymphocytes, histamine H_2 blockers, and various products of immune cells (Drews 1985).

Host-Vector Interactions

Vector-borne diseases, such as leishmaniasis and malaria, depend upon the frequency and success of host/vector interactions for transmission. Vector contact with an infected host, successful uptake of infected fluid, and, after a given period of time, subsequent contact and feeding upon a susceptible host, are implicit in the process of transmission. These components of arthropod-borne diseases have received increasing attention (Balashov 1984, Day & Edman 1983, 1984a, 1984b, Day et al. 1983, Edman et al. 1972, 1974). The numerical abundance of the vector population, their longevity under conditions favorable to parasite development, and the affinity of the arthropod for vertebrates capable of harboring the parasite are among the more important characteristics relevant to the transmission process (Reeves 1971).

Host behavior can clearly play an important role in determining whether a parasite has an opportunity to be transmitted by an arthropod (Day & Edman 1984b, Edman et al. 1974, Waage & Nondo 1982, Walker & Edman 1985 1986). In some instances modification of host behavior may be induced by a parasite, resulting in an increased probability of pathogen transmission (Dawkins 1982). Rodent defensive behavior normally prevents mosquitoes from successfully feeding upon them, thereby eliminating the possibility of disease transmission. However, lab mice infected with Plasmodium berghei, P. chabaudi, or P. yoelii exhibited periods of reduced defensive behavior which corresponded to maximum gametocyte infectivity. This period did not

necessarily correspond with maximum parasitemia (Day & Edman 1983a). Dye and Hasibeder (1986) examined the dynamics of vector-borne diseases when non-random host/vector contact occurred.

Arthropod behavior may also be manipulated by the parasite in such a way that transmission of the pathogen is facilitated. Two opposing processes which affect feeding success, vector contact and impairment of feeding processes, are known to promote disease transmission.

Vector contact with the host is fraught with risks, primarily due to host defensive behavior. Rapid feeding minimizes the period spent by the vector on the host without sacrificing maximum nutrient uptake; this presumably increases vector survival (Daniel & Kingsolver 1983, Mellink 1981). Arthropods minimize host contact by releasing antihemostatic salivary components that inhibit platelet aggregation and enhance blood location (Ribiero et al. 1984). Numerous vector-borne parasites inhibit host hemostasis. This inhibition contributes to blood-finding success (Rossignol et al. 1985). Examples of alteration of host hemostasis include thrombocytopenia resulting from malaria, dengue, African trypanosomiasis, leishmaniasis, and babesiosis (Allen et al. 1975, Davis 1982, Halstead 1984), hemorrhagic disorders caused by Rift Valley fever virus, and vasodilation resulting from infection with Rocky Mountain spotted fever and endemic typhus (Rossignol et al. 1985).

Alternatively, <u>impairment</u> of processes affecting feeding may also facilitate pathogen transmission. Numerous studies have shown that parasites can cause pathology to the vector. Arthropods

infected with Leishmania (Beach et al. 1985), Plasmodium (Rossignol et al. 1984 1986), and trypanosomes (Anez & East 1984, Jenni et al. 1980) probe more frequently than their uninfected counterparts. Mosquitoes infected with La Crosse virus have an impaired ability to feed, and therefore may contact more hosts during a single gonotrophic period (Grimstad et al. 1980). A combination of impaired probing ability with host defensive behaviors may extend feeding time and/or increase the number of hosts contacted by an infected insect.

Chapter II

INTERACTIONS BETWEEN *PLASMODIUM YOELII* AND *LEISHMANIA MEXICANA AMAZONENSIS* IN *LEISHMANIA* SUSCEPTIBLE BALB/C MICE

Introduction

Human leishmaniasis is a protozoan disease with a wide spectrum of clinical manifestations (Turk & Bryceson 1971, Belehu et al. 1980). Three primary forms of human disease have been described. Each of these forms may exhibit a variety of symptoms (Molyneux & Ashford 1983), ranging from inapparent infections (Pampiglione et al. 1974) to fulminating cases with fatal sequelae (Hill 1986). The clinical symptoms that develop in a given individual reflect properties of the parasite involved as well as intrinsic host features, such as genetic background and immunological competence (Blackwell & Ulczak 1984, Hill 1986, Semprevivo et al. 1981).

Evidence suggests that clinically apparent cases of leishmaniasis, no matter how severe the symptoms are once these cases become established, comprise only a small fraction of all actual infections (Pampiglione et al. 1974, Fuller et al. 1976 1979). Serological evidence has shown that cryptic *L. donovoni* infections are widespread in Ethiopia (Ayele & Ali 1984, Fuller et al. 1976, 1979) and Italy

(Pampiglione et al. 1974), yet clinically evident cases of visceral leishmaniasis are quite rare in these countries. American cutaneous leishmaniasis may occur as a cryptic infection (Gustafson et al. 1985), and L. braziliensis has been shown to remain latent for many years without the development of clinical symptoms (Walton et al. 1973).

The factors which determine whether or not an individual will develop clinically evident leishmaniasis have not been conclusively determined. Laboratory studies have shown that the genetic background of the host is important. Different strains of inbred mice vary greatly in their response to Leishmania. Some strains are completely resistant to the parasite while others are acutely susceptible (Howard et al. 1980, Perez et al. 1979, Semprevivo et al. 1981). The immunological competence of the host also affects disease outcome (Liew et al. 1982). The development of leishmaniasis at the site of inoculation and in metastatic visceral and cutaneous foci is influenced by host immunity. Failure of immune function has been suggested as a primary factor that may lead to the development of disseminated forms of leishmaniasis (Hill 1986).

Any factor that has the capacity to alter immunological function could theoretically affect the course of leishmaniasis. Pampiglione et al. (1974) suggested that stress, i.e. famine or epidemic malaria, could affect the competence of the immune system. This could transform an inapparent infection into a clinically evident case of leishmaniasis. Walton et al. (1973) proposed that tuberculosis could upset immunological function, thereby precipitating the

invasion of mucosal tissue by L. braziliensis after many years of asymptomatic infection.

Outbreaks of visceral leishmaniasis are common following epidemics of malaria (Pampiglione et al. 1974). Malaria coincides geographically with leishmaniasis in many parts of the tropics. Numerous studies have shown that infection with malaria can result in the enhancement of concomitant infections, presumably via an immunosuppressive mechanism (Greenwood 1974, Khansari et al. 1981, Killick-Kendrick & Peters 1978, Krettli 1977, Lelchuk & Playfair 1980, Lwin et al. 1982). Malaria-mediated immunosuppression has been implicated in the development of Burkitt`s lymphoma (Salaman 1970), and loss of cellular-mediated immunity due to malaria can lead to reactivation of herpes simplex viruses (Cook 1985).

In the only study on concomitant leishmaniasis and malaria, Adler (1954) examined interactions between P. berghei and L. infantum in the golden hamster, Mesocricetus auratus. Animals previously infected with L. infantum were somewhat resistant to the normally lethal P. berghei. There was no evidence that either infection was enhanced in dually infected animals.

The purpose of this study was to determine whether concurrent infection with malaria would result in enhanced leishmaniasis in highly susceptible mice. Interactions between Plasmodium yoelii and Leishmania mexicana were examined in BALB/c mice. These mice are a satisfactory model for progressive cutaneous leishmaniasis (Perez 1983). The interval between the inoculation of each parasite was

varied. This allowed me to examine the role of the timing of inoculation of each parasite on the course of the concomitant pathogen.

Materials and Methods

Animals

Female BALB/c mice were obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts. Eight-week-old female mice weighing 25-30 g were used for all experiments.

Malaria

The 17x strain of *Plasmodium yoelii* was used. It was kindly provided by Dr. J. Day. It is maintained in our laboratory by weekly passage of infected blood in BALB/c mice. Inocula were prepared from red blood cells (RBC) collected in phosphate-buffered saline (PBS) from the tail of an infected mouse. The percent of infected RBC were determined from Giemsa-stained thin blood smears. A hemocytometer was used to determine the actual number of RBC per unit volume. Blood was then diluted with PBS so that each mouse received 1×10^6 infected RBC i.p. in 0.2 ml. The course of infection was monitored by determining *P. yoelii* parasitemia in Giemsa-stained blood smears (the percent of RBC infected with *P. yoelii* is presented as the "index of malaria" in all Figures). Blood smears were prepared every 2 days until no parasites were found over a 6 day period. All mice were monitored once a week thereafter to check for possible relapses of the *Plasmodium*

Leishmaniasis

The Walter Reed strain 227 of Leishmania mexicana amazonensis was used. The source and history of this isolate have been described (Nolan et al. 1984). Amastigotes were obtained from donor mice 7-10 weeks after subcutaneous inoculation of 2×10^6 amastigotes into the right-rear footpad. The doner was sacrificed and the infected foot amputated, surface sterilized with 70% ETOH, and homogenized in a Ten-Broeck glass tissue grinder containing 3-4 ml PBS. Debris was allowed to settle, then the supernatant pipetted off for use. Amastigote viability was determined using the method of Hodgkinson and Herman (1980) and the number of parasites per unit volume calculated using a Petroff-Housser bacterial cell counting chamber. The solution was adjusted with PBS to obtain a final concentration of either 1×10^6 or 2×10^6 viable amastigotes in 10 microliters PBS. Mice were infected by subcutaneously inoculating 10 microliters of the appropriate solution into the ventral surface of the right-rear-footpad. The thickness of the infected footpad was measured once a week using a direct-reading vernier caliper. Measurements commenced 1 week prior to the inoculation of L. mexicana and continued for at least 10 weeks. Lesion diameter was expressed as the thickness of the infected footpad in millimeters minus the thickness of the contralateral uninfected footpad (the mean adjusted lesion diameter is expressed as the "Index of Leishmaniasis" in all Figures).

Experimental Design

An initial experiment was designed to test our experimental protocol for precision and reliability. Three groups of 30 mice each were infected with either 1 x 10^6 L. mexicana amastigotes, 2 x 10^6 L. mexicana amastigotes, or 1 x 10^6 P. yoelii infected RBC. Mean values were recorded and plotted.

Five additional experiments were conducted. The interval between the inoculation of the two parasites was varied in each experiment, as follows:

1) P. yoelii followed by L. mexicana in 2 days (1 x 10^6 amastigotes).
2) P. yoelii followed by L. mexicana in 3 weeks (2 x 10^6 amastigotes).
3) P. yoelii followed by L. mexicana in 20 weeks (2 x 10^6 amastigotes).
4) L. mexicana (2 x 10^6 amastigotes) followed by P. yoelii in 3 weeks.
5) L. mexicana (2 x 10^6 amastigotes) followed by P. yoelii in 12 weeks.

Four groups of 10 mice each were used for each of the 5 experiments. These groups consisted of: 1) uninfected mice, 2) P. yoelii infected mice, 3) L. mexicana infected mice, and 4) P. yoelii and L. mexicana infected mice. Mean P. yoelii parasitemia rate and mean L. mexicana lesion size were recorded for each group of mice in each experiment.

Results

Consistent P. yoelii and L. mexicana infections were produced in the initial experiment (Figs. 1 & 2). Based on these results I believed that similar precision and consistency would be expected during experiments on dual infections.

Results from the 5 experiments examining interactions between parasites are shown in Figs. 3-7. Peak malaria infections with 10-15% infected RBC`s occurred in mice inoculated with P. yoelii only. None of these mice died. When P. yoelii was preceded by L. mexicana, the malaria infection was both enhanced and prolonged (Figs 3, 4, & 6). Up to 50% of the mice in these groups died.

Two different doses of L. mexicana were used to inoculate mice. Regardless of the dose, L. mexicana infections were more severe in those mice where P. yoelii was inoculated at or around the same time as L. mexicana compared to mice infected with L. mexicana only (Figs. 3, 4, & 5). This enhancement of the leishmaniasis began within 1-2 weeks following the elimination of P. yoelii from the peripheral blood and continued for the duration of the infection. When P. yoelii was inoculated 20 weeks prior to L. mexicana no enhancement of the leishmaniasis occurred (Fig. 7).

Figure 1. Course of Plasmodium yoelii in 30 BALB/c mice. Mice were infected on Day 0 (+/- SE).

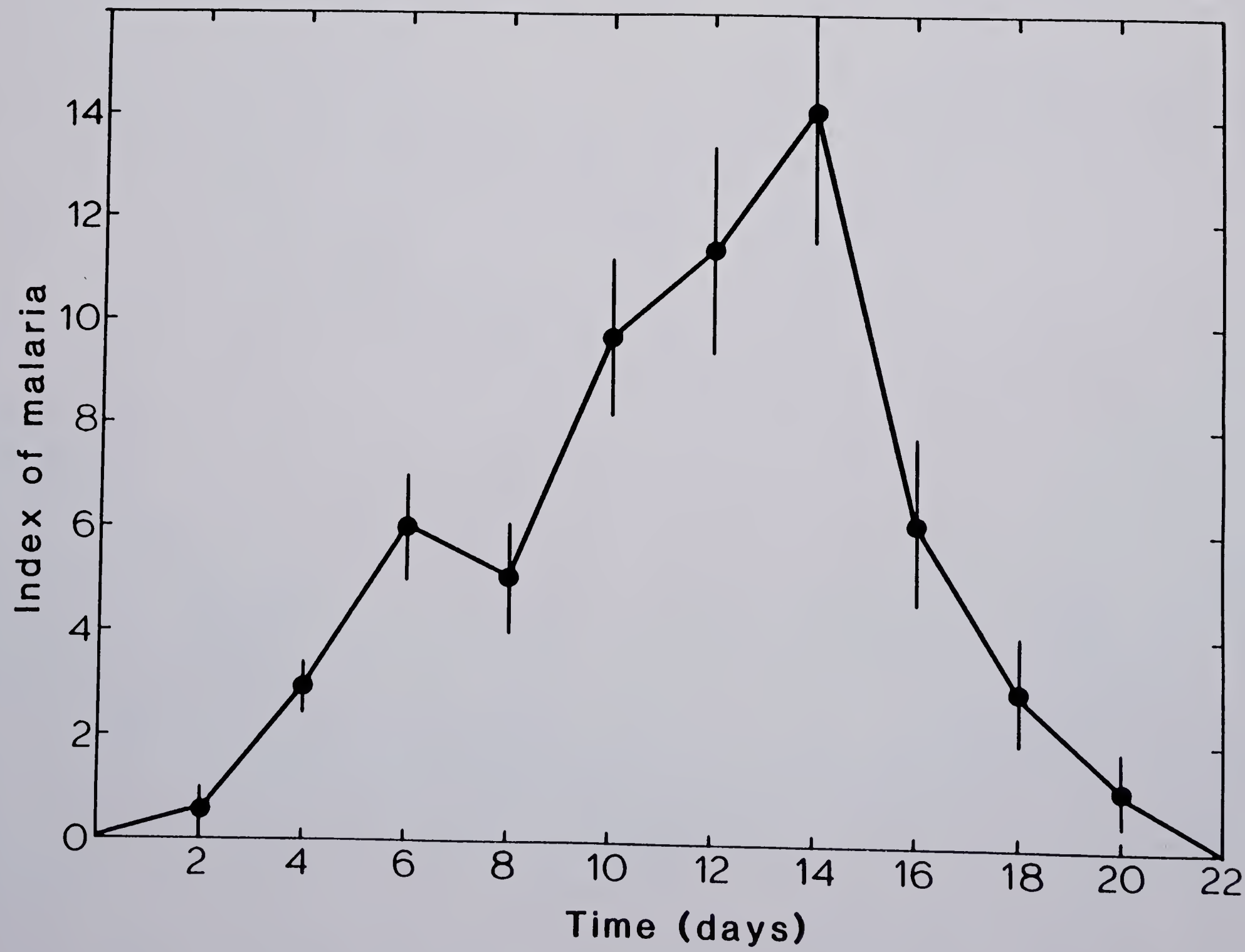

Index of malaria
0
2
4
6
8
10
12
14
2
4
6
8
10
12
14
16
18
20
22
Time (days)

Figure 2. Course of *Leishmania mexicana* in BALB/c mice infected with 1×10^6 or 2×10^6 amastigotes. Mice were infected on Week 0 (+/- SE).

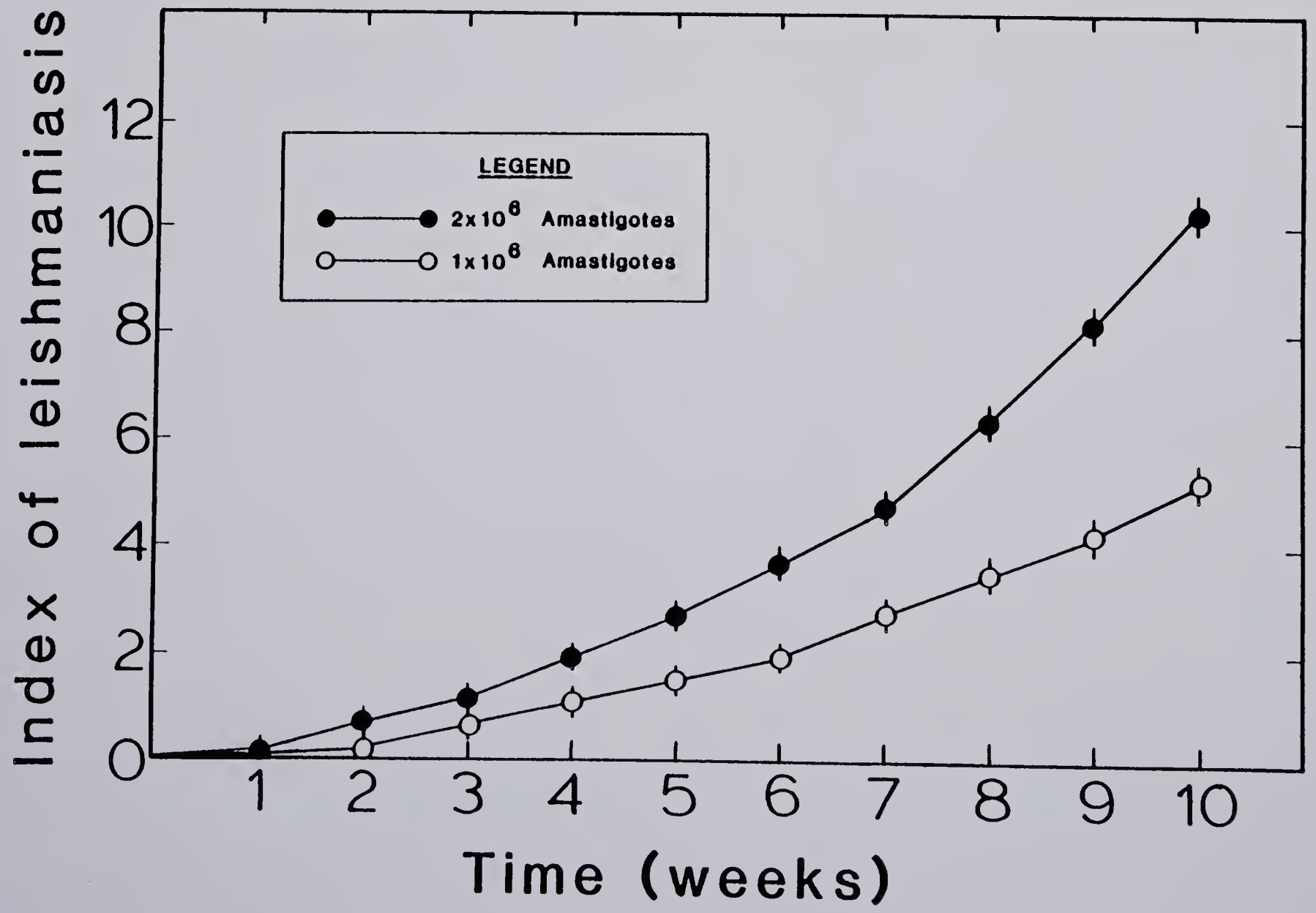

Index of leishmaniasis
LEGEND
2x10^6 Amastigotes
1x10^6 Amastigotes
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
9
10
Time (weeks)

Figure 3. Interactions between Plasmodium yoelii and Leishmania mexicana in BALB/c mice. L. mexicana followed by P. yoelii in two days (+/- SE).

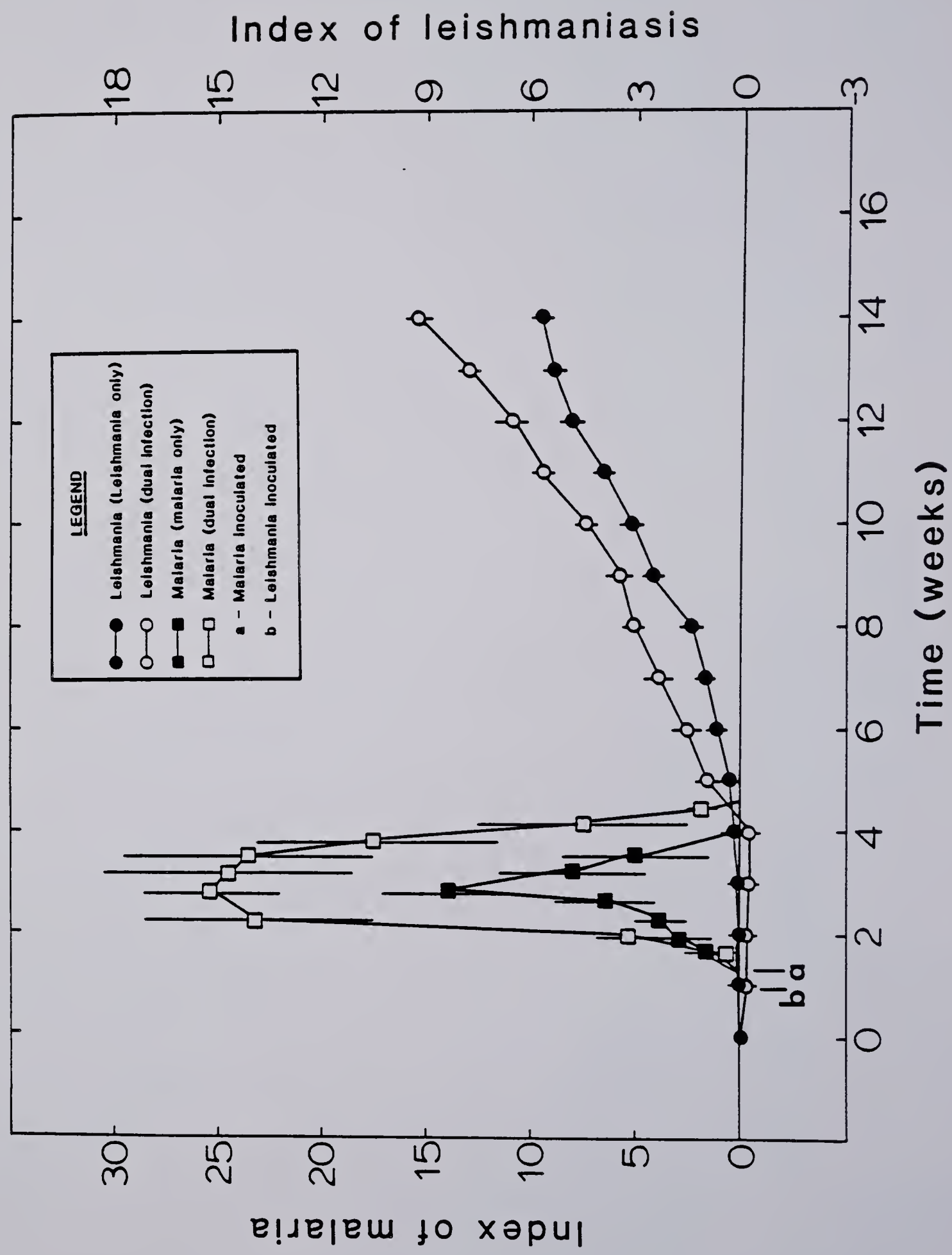

Index of leishmaniasis
18
15
12
9
6
3
0
-3
LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (malaria only)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated
8
10
12
14
16
Time (weeks)
0
2
4
6
b
a
Index of malaria
30
25
20
15
10
5
0

Figure 4. Interactions between Plasmodium yoelii and Leishmania mexicana in BALB/c mice. L. mexicana followed by P. yoelii in three weeks (+/- SE).

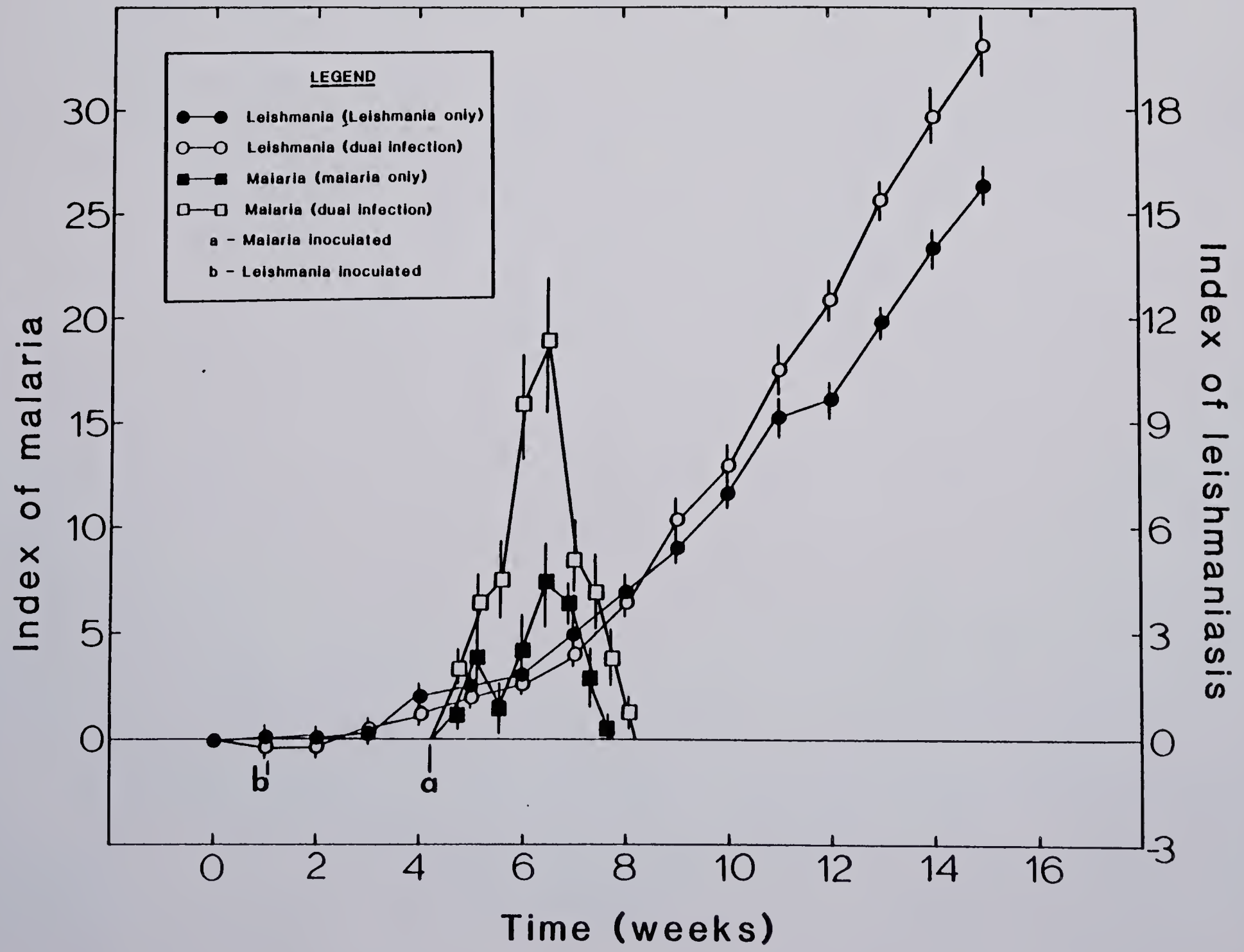

LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (malaria only)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated
Index of malaria
Index of leishmaniasis
Time (weeks)
30
25
20
15
10
5
0
18
15
12
9
6
3
0
-3
0
2
4
6
8
10
12
14
16
b
a

Figure 5. Interactions between Plasmodium yoelii and Leishmania mexicana in BALB/c mice. P. yoelii followed by L. mexicana in three weeks (+/- SE).

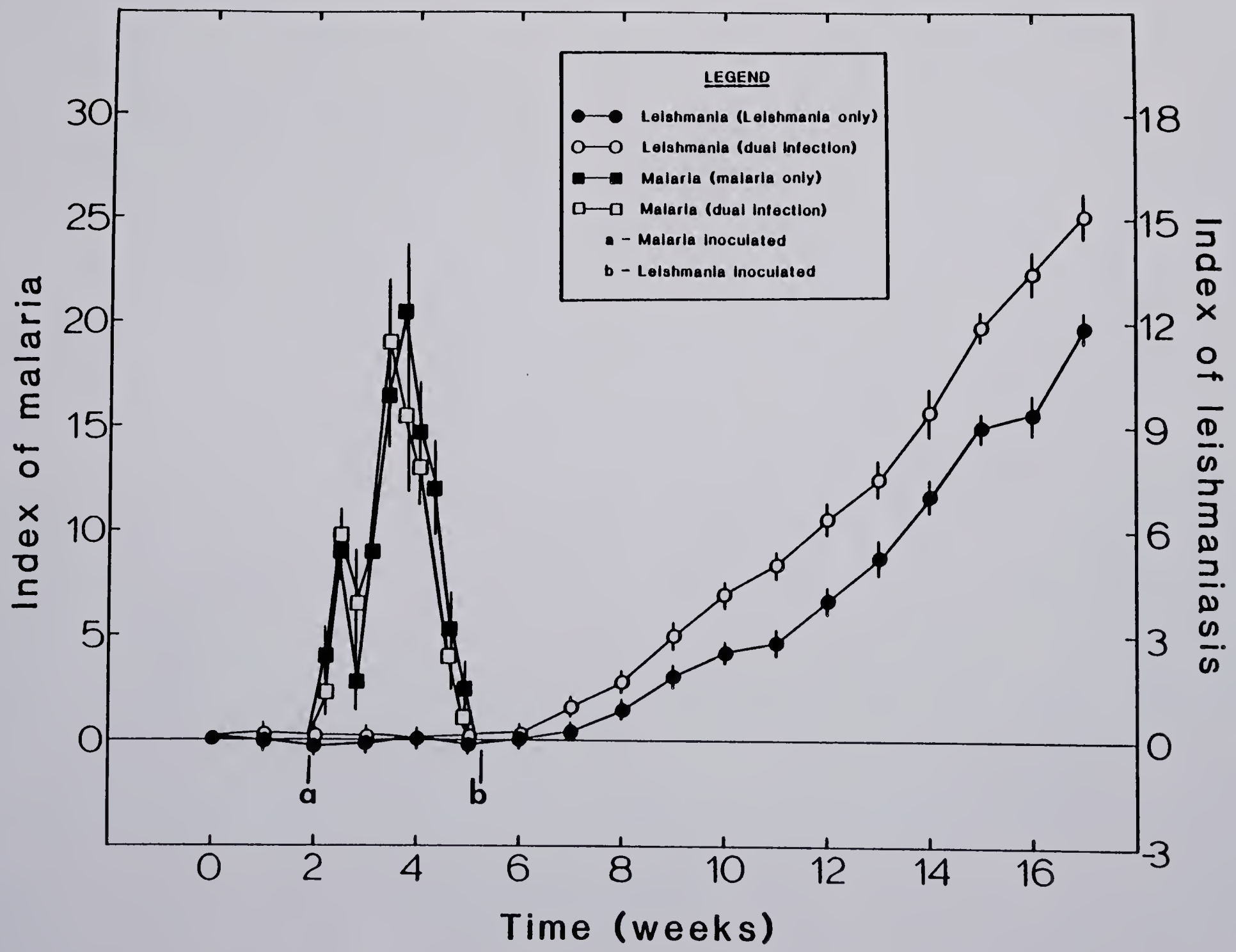

LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (malaria only)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated
Index of malaria
Index of leishmaniasis
Time (weeks)
30
25
20
15
10
5
0
18
15
12
9
6
3
0
-3
0
2
4
6
8
10
12
14
16
a
b

Figure 6. Interactions between Plasmodium yoelii and Leishmania mexicana in BALB/c mice. L. mexicana followed by P. yoelii in twelve weeks (+/- SE).

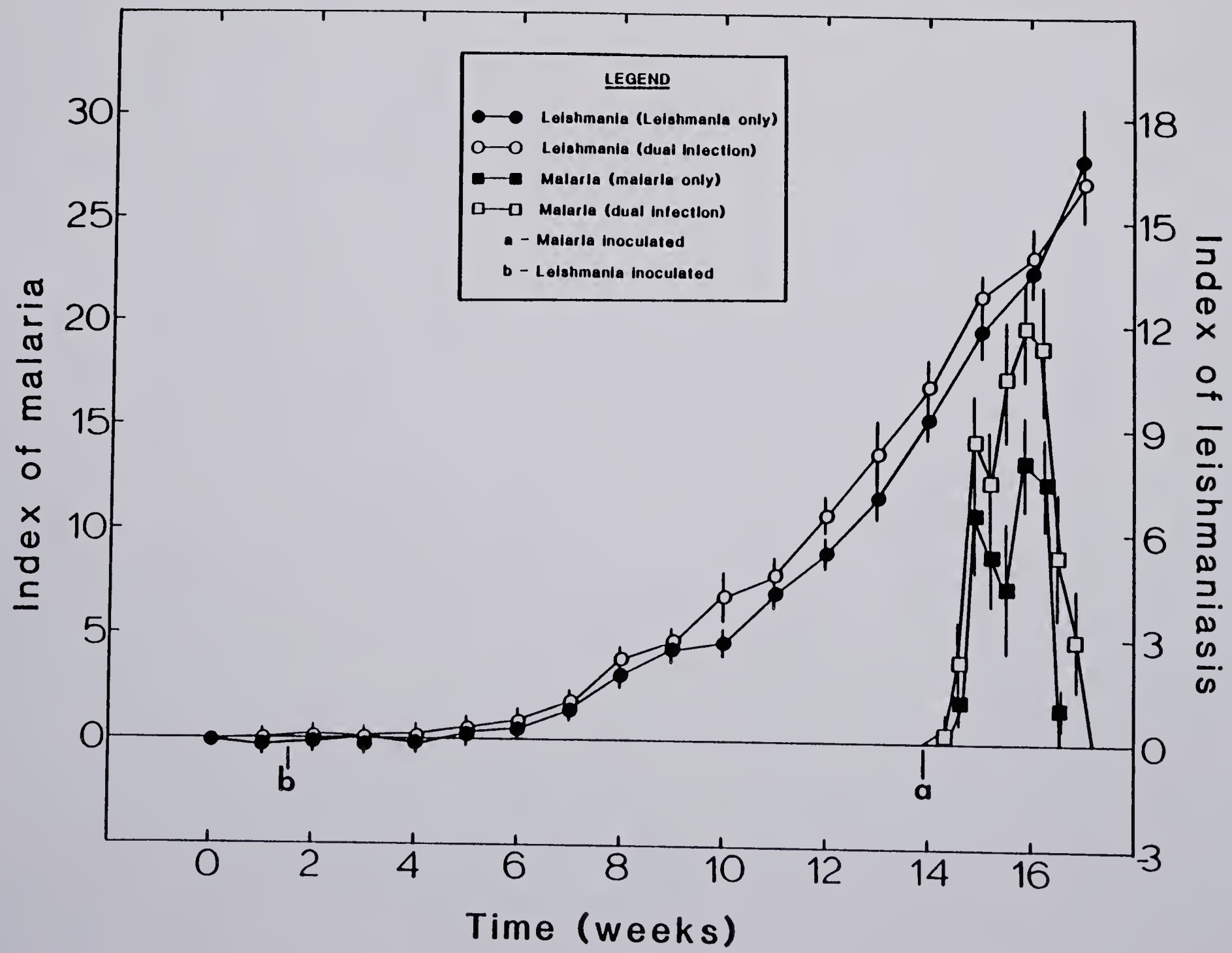
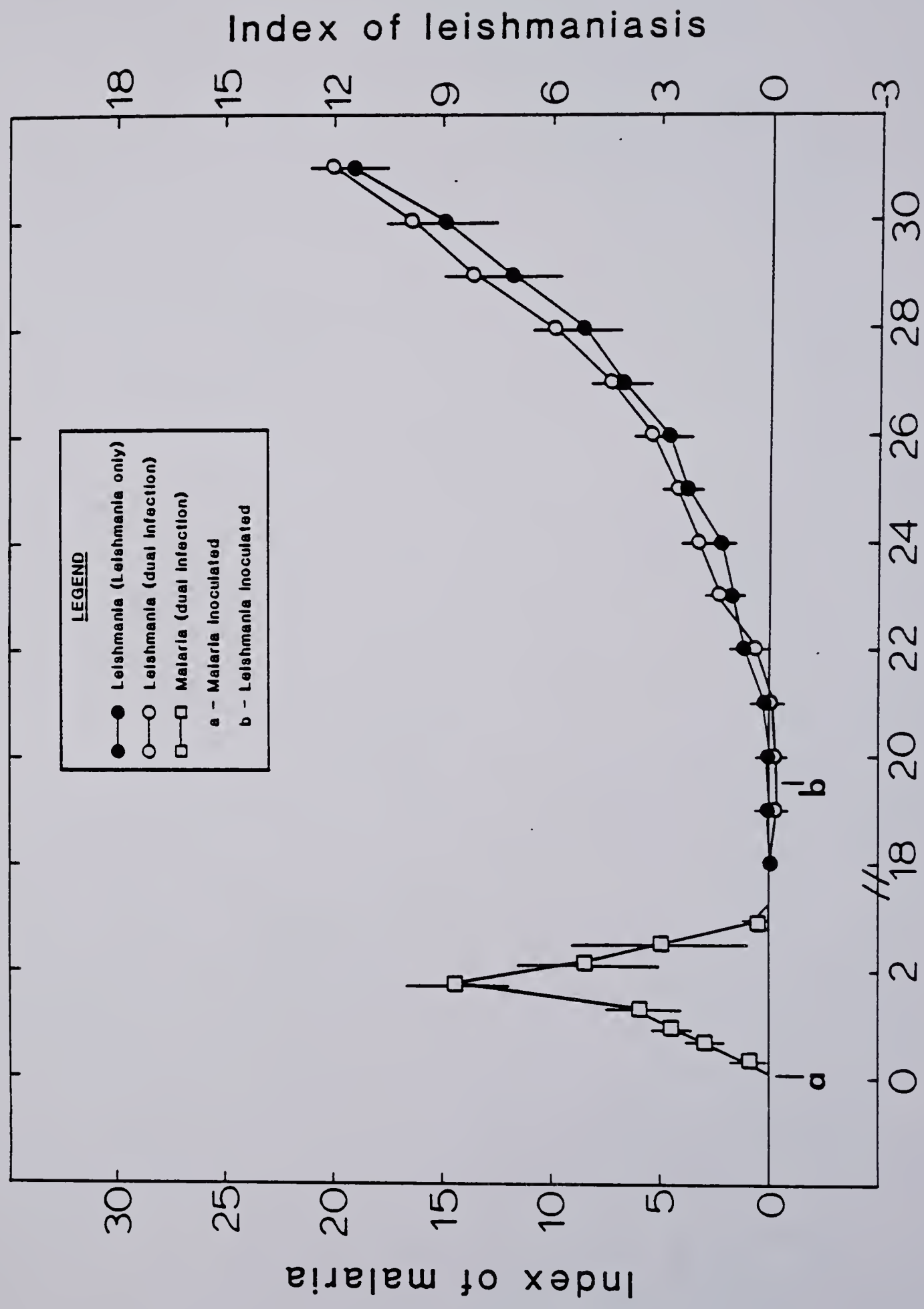

LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (malaria only)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated
Index of malaria
Index of leishmaniasis
Time (weeks)
30
25
20
15
10
5
0
18
15
12
9
6
3
0
-3
0
2
4
6
8
10
12
14
16
a
b

Figure 7. Interactions between Plasmodium yoelii and Leishmania mexicana in BALB/c mice. P. yoelii followed by L. mexicana in twenty weeks (+/- SE).


Index of leishmaniasis
18
15
12
9
6
3
0
-3
LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated
22
24
26
28
30
Time (weeks)
Index of malaria
30
25
20
15
10
5
0
0
2
18
20
a
b

Discussion

The primary objective of this research was to determine if concomitant infection with malaria would result in more acute leishmaniasis in the susceptible BALB/c mouse. Results clearly demonstrated (see Fig. 3) that not only did malaria enhance the course of cutaneous leishmaniasis, but that fatality rates of up to 50% could be ascribed to the malaria in those mice infected with both L. mexicana and the normally non-lethal P. yoelii. These findings suggest that in those regions of the world where malaria coincides geographically with leishmaniasis, clinical incidence and severity of the diseases in humans also could be influenced by concomitant infections.

Under natural conditions the two infections often may not initiate at the same time. With this in mind, experiments were designed to determine the effect if the malaria or leishmaniasis were initiated well before or after the other infection. Results presented in Figs. 4 and 5 clearly demonstrate that the leishmanial infection is enhanced if the malaria is introduced 3 weeks before or after the Leishmania inoculation. Similarly, the malaria was enhanced in all cases of concomitant infection (see Figs. 3, 4, & 6). Somewhat surprisingly, enhancement of P. yoelii parasitemia was minimal when that parasite was inoculated during advanced L. mexicana infection (Fig. 5). Existing knowledge indicates that cell-mediated immunity is suppressed during this stage of the infection (Howard et al. 1981). Why the malarial infection was not affected more significantly is not known. The most pronounced influence of leishmaniasis upon the

malaria occurred when the former was inoculated only a few days before the latter (see Fig. 3). However, even when the malarial infection was initiated 3 weeks before the leishmaniasis it had a strong influence on the leishmanial infection. In both these situations the concomitant infection strongly influenced the capacity of the host to resist the second infection, yet the original infections were either subpatent or apparently eliminated. Documentation of the effect of concomitant infections in humans may require not only immediate examination for dual infections but significant follow-up and knowledge of the patient's history.

Although the mechanism by which each of these parasitic infections enhances the other is not known, it has been established that each elicits immune suppressor activity. In many areas of the world where multiple parasitic infections in humans may be the rule rather than the exception, specific and nonspecific immune suppression resulting from previous, concurrent, or subpatent infections may be of considerable influence to chemotherapeutic and immunologic intervention.

These results are in direct contrast to those obtained by Adler (1954), who found that infection with L. infantum in the golden hamster protected against the normally lethal P. berghei. Existing knowledge on the role of cell-mediated immunity during malaria and leishmaniasis indicates that suppression of immune function should lead to enhancement, not suppression, of concomitant infections. Disease enhancement may be a general phenomenon resulting from concomitant malarial - leishmanial infections.

Chapter III

INTERACTIONS BETWEEN *PLASMODIUM YOELII* AND *LEISHMANIA MEXICANA AMAZONENSIS* IN *LEISHMANIA* RESISTANT C/57 MICE

Introduction

Human disease due to infection with *Leishmania mexicana* normally consists of a single lesion which spontaneously heals (Perez et al. 1978). Recovery is usually accompanied by the development of delayed hypersensitivity response to leishmanin as well as immunity to reinfection by the parasite.

Various strains of inbred mice are commonly used as experimental hosts for *L. mexicana* (Barral et al. 1983, Neal & Hale 1983, Perez et al. 1978, 1979). Different strains of mice vary greatly in their responses to *L. mexicana*. Some mice (e.g., C/57 strain) are resistant to *L. mexicana* while others (e.g., BALB/c strain) are acutely susceptible to the parasite (Perez et al. 1979).

Numerous studies have shown that infection with malaria can result in the enhancement of concomitant infections, presumably via an immunosuppressive mechanism (Greenwood 1974, Khansari et al. 1981, Killick-Kendrick & Peters 1978, Krettli 1977, Lelchuk & Playfair 1980, Lwin et al. 1982). The purpose of this study was to determine

whether concurrent infection with malaria would result in enhanced leishmaniasis in a *Leishmania*-resistant mouse strain.

Interactions between *Plasmodium yoelii* and *L. mexicana amazonensis* were examined in C/57 mice. The interval between the inoculation of each parasite was varied. Similar experiments were previously conducted in *Leishmania*-susceptible BALB/c mice; however, the presentation of *L. mexicana* in BALB/c mice does not reflect the normal course of the parasite in humans. In this respect C/57 mice provide a more realistic model for studying interactions between malaria and leishmaniasis.

Materials and Methods

Animals

Female C/57 mice were obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts. Eight-week-old mice weighing 25-30 g were used for all experiments.

Malaria

Techniques for inoculating and quantifying *P. yoelii* have been described (Chapter 2).

Leishmaniasis

Techniques for inoculating and quantifying *L. mexicana amazonensis* have been described (Chapter 2).

Experimental Design

An initial experiment was designed to test the experimental protocol for the inoculation and quantification of L. mexicana in C/57 mice. Two groups of 30 mice each were infected with either 1 x 10^6 or 2 x 10^6 L. mexicana amastigotes. Mean lesion diameters were recorded.

Five additional experiments were conducted. The interval between the inoculation of P. yoelii and L. mexicana was varied in each experiment, as follows:

1) P. yoelii followed by L. mexicana (1 x 10^6 amastigotes) in 2 day.
2) P. yoelii followed by L. mexicana (2 x 10^6 amastigotes) in 3 weeks.
3) P. yoelii followed by L. mexicana (2 x 10^6 amastigotes) in 16 weeks.
4) L. mexicana (2 x 10^6 amastigotes) followed by P. yoelii in 3 weeks.
5) L. mexicana (2 x 10^6 amastigotes) followed by P. yoelii in 12 weeks.

Four groups of 10 mice each were used for each of the 5 experiments. These groups consisted of: 1) uninfected mice, 2) P. yoelii infected mice, 3) L. mexicana infected mice, and 4) P. yoelii and L. mexicana infected mice. Mean P. yoelii parasitemia rate and mean L. mexicana lesion size were recorded for each group of mice in each experiment.

Results

Consistent *Leishmania* infections were produced with both dosages of *L. mexicana* in the initial experiment (Fig. 8). Results from experiments examining interactions between parasites are shown in Figures 9-13. The *P. yoelii* infection was significantly enhanced in those instances where *L. mexicana* was inoculated prior to the malaria (Figs. 9, 10, & 12). *L. mexicana* lesions were greater in mice where the malaria was inoculated before or slightly after the leishmaniasis (Figs. 9, 10, 11, & 13).

Figure 8. Course of Leishmania mexicana in C/57 mice infected with 1 x 10^6 or 2 x 10^6 amastigotes. Mice were infected on Week 0 (+/- SE).

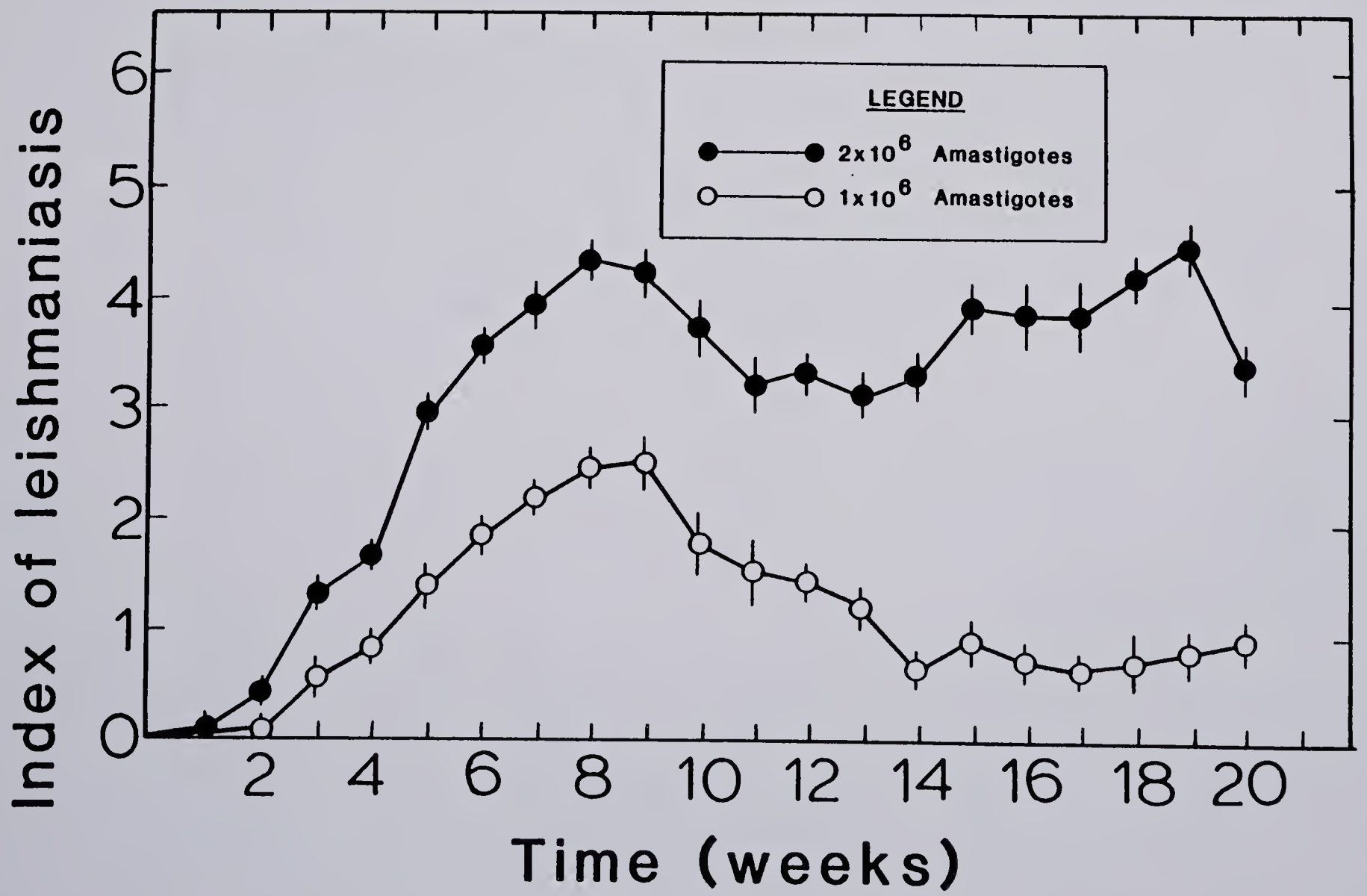

LEGEND
2x10^6 Amastigotes
1x10^6 Amastigotes
Index of leishmaniasis
0
1
2
3
4
5
6
2
4
6
8
10
12
14
16
18
20
Time (weeks)

Figure 9. Interactions between Plasmodium yoelii and Leishmania mexicana in C/57 mice. L. mexicana followed by P. yoelii in two days (+/- SE).

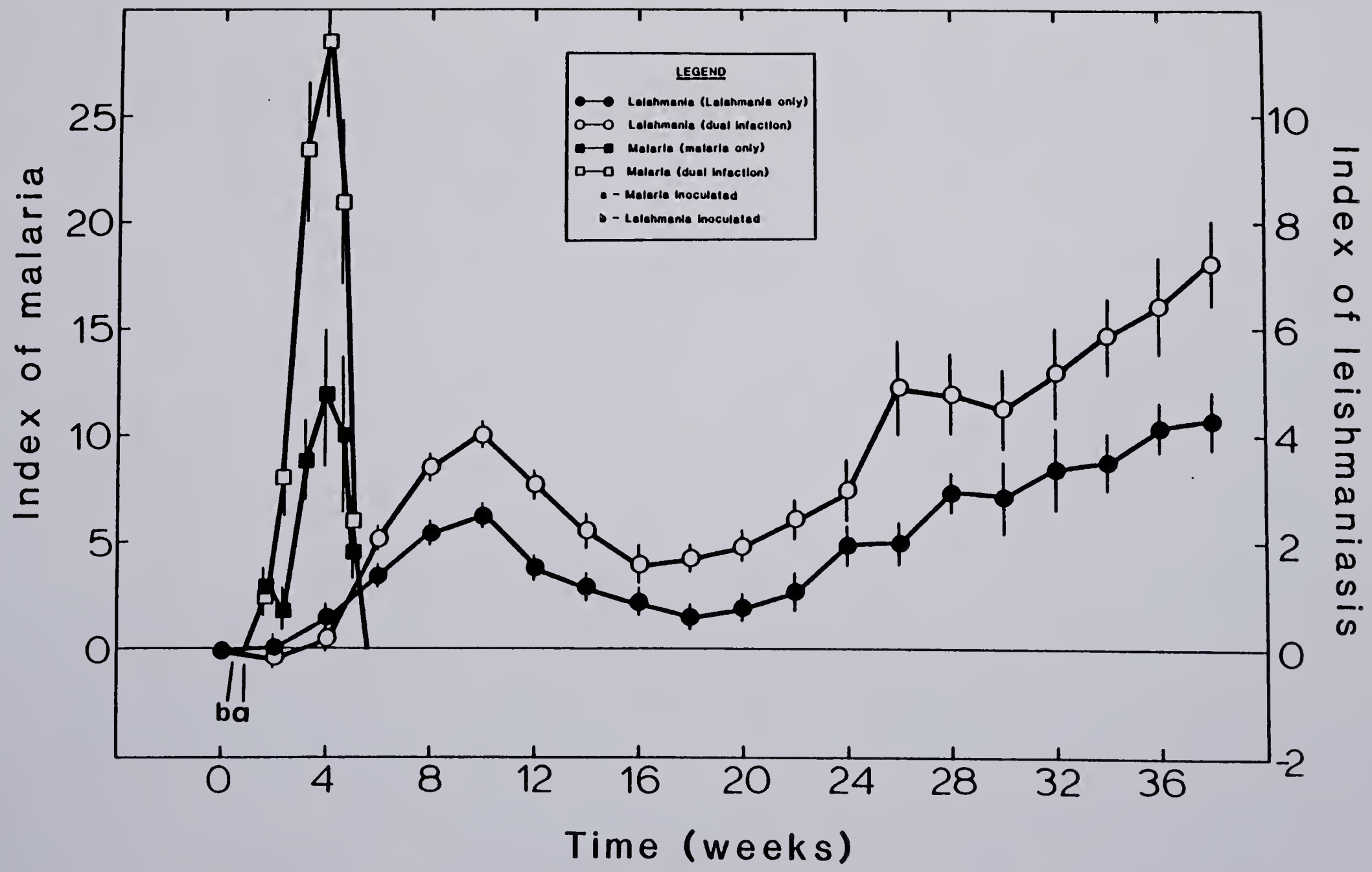

LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (malaria only)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated
Index of malaria
Index of leishmaniasis
25
20
15
10
5
0
10
8
6
4
2
0
-2
ba
0
4
8
12
16
20
24
28
32
36
Time (weeks)

Figure 10. Interactions between Plasmodium yoelii and Leishmania mexicana in C/57 mice. L. mexicana followed by P. yoelii in three weeks (+/- SE).

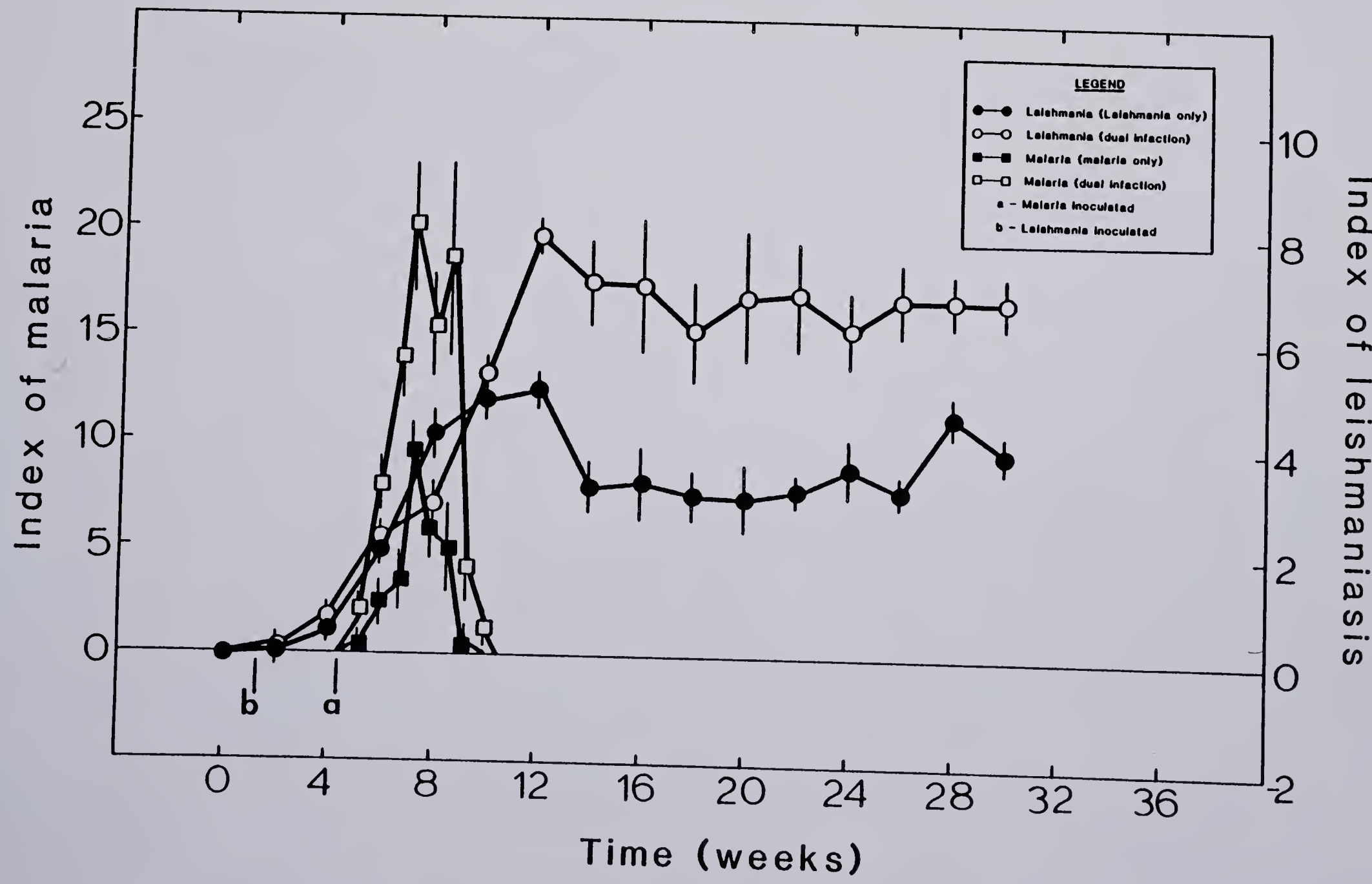

LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (malaria only)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated
Index of malaria
Index of leishmaniasis
Time (weeks)
0
4
8
12
16
20
24
28
32
36
25
20
15
10
5
0
10
8
6
4
2
0
-2
b
a

Figure 11. Interactions between Plasmodium yoelii and Leishmania mexicana in C/57 mice. P. yoelii followed by L. mexicana in three weeks (+/- SE).

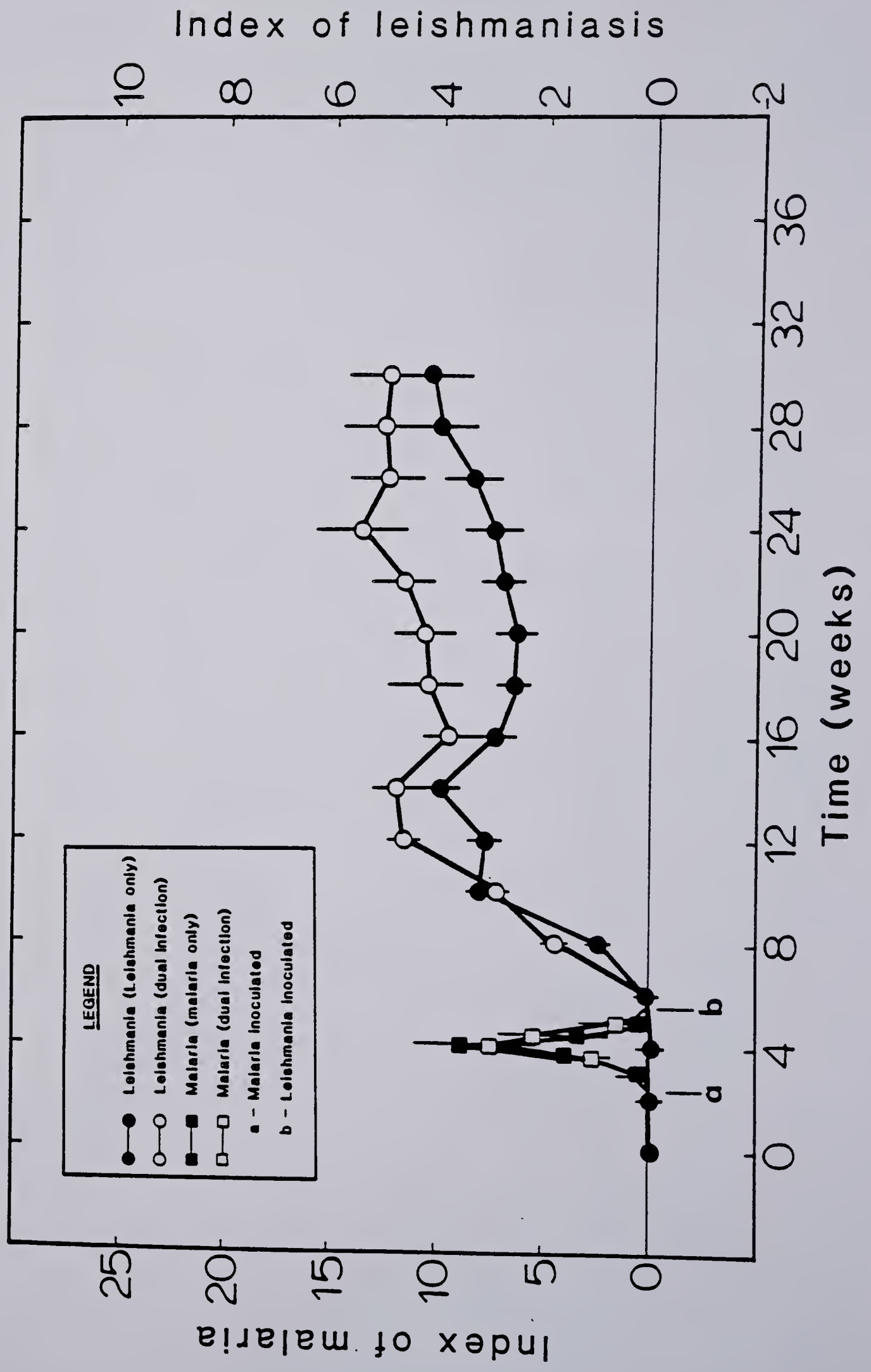

Index of leishmaniasis
10
8
6
4
2
0
-2
36
32
28
24
20
16
12
8
4
0
Time (weeks)
Index of malaria
25
20
15
10
5
0
a
b
LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (malaria only)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated

Figure 12. Interactions between Plasmodium yoelii and Leishmania mexicana in C/57 mice. L. mexicana followed by P. yoelii in twelve weeks (+/- SE).

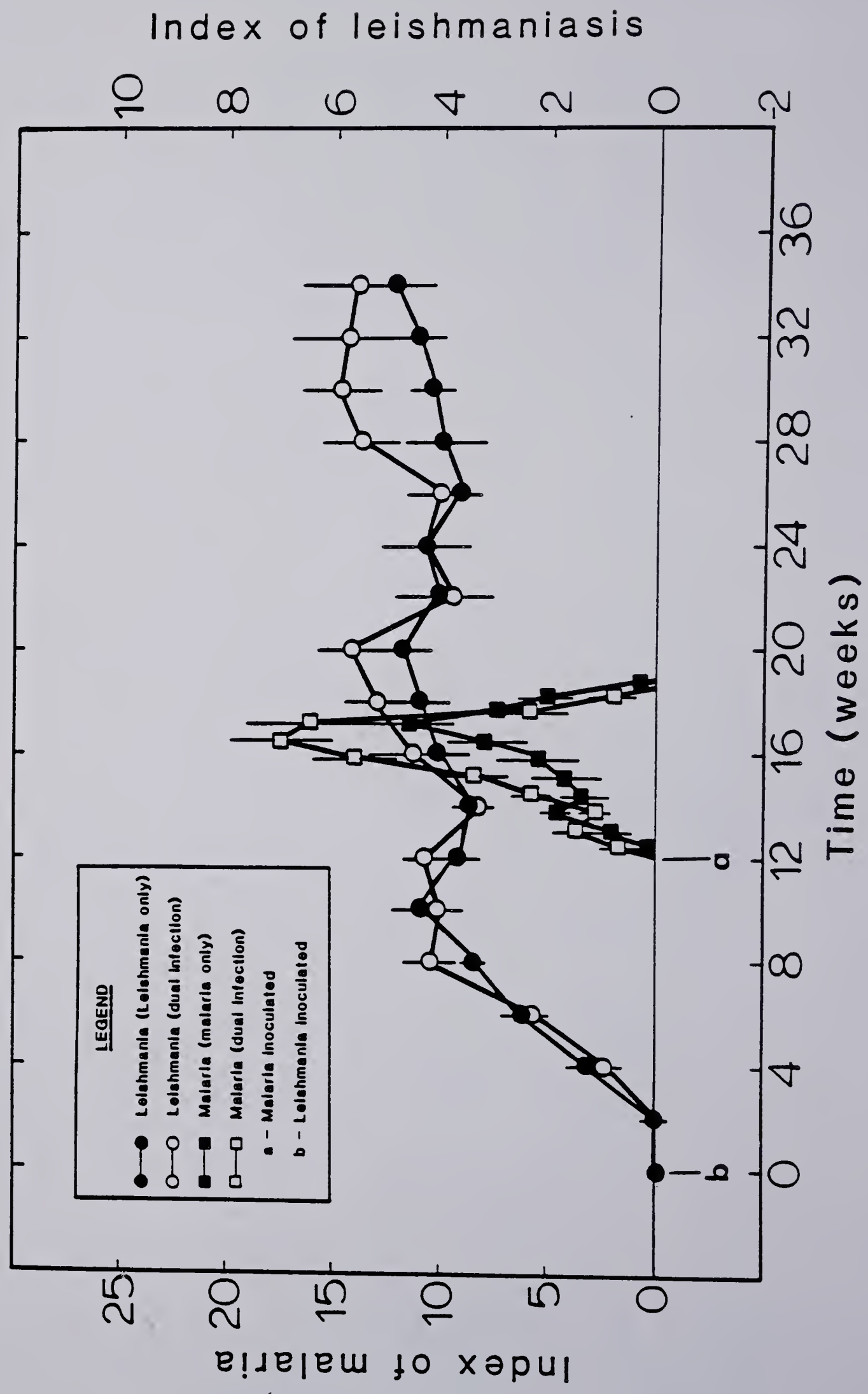
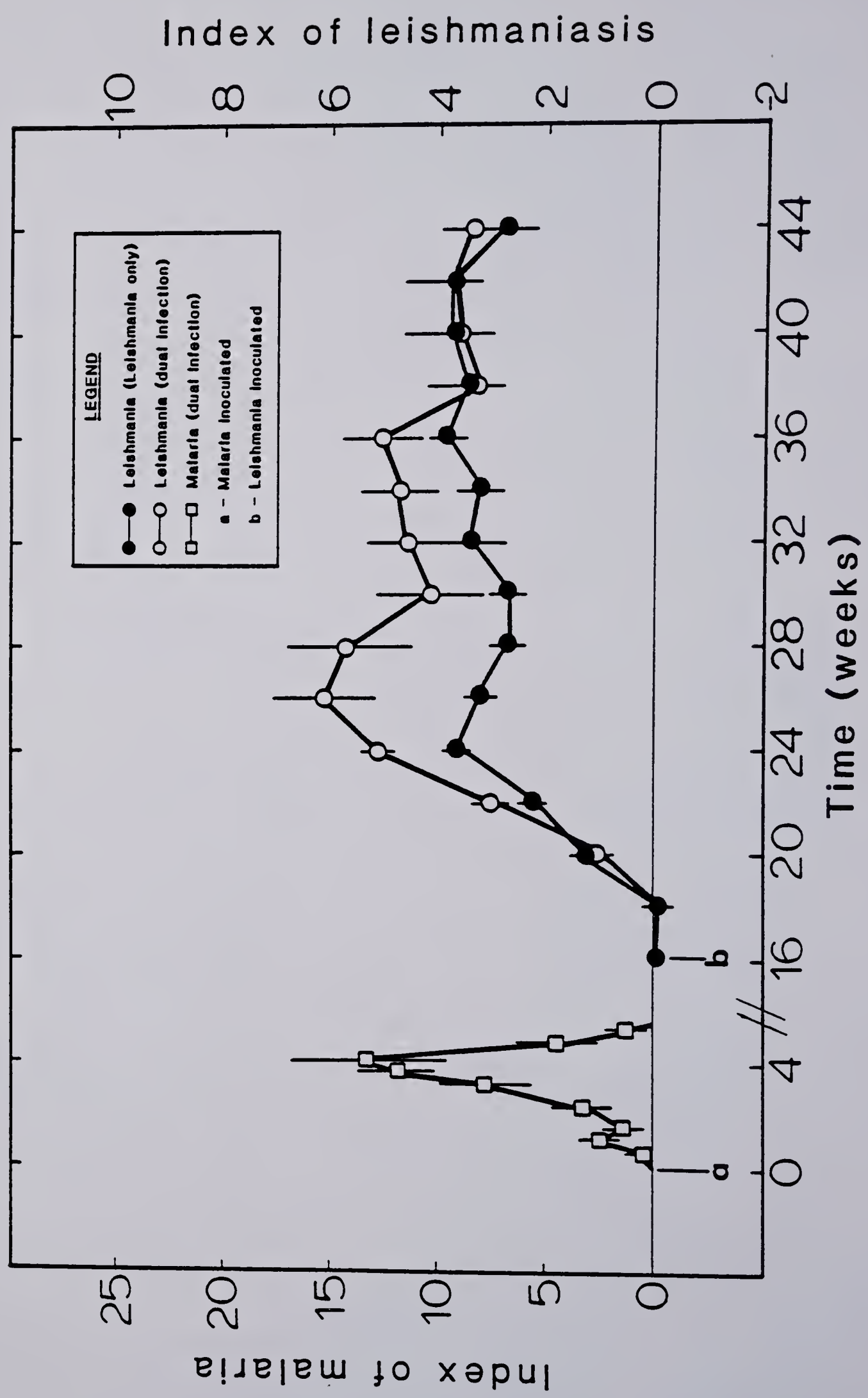

Index of leishmaniasis
10
8
6
4
2
0
-2
36
32
28
24
20
16
12
8
4
0
Time (weeks)
a
b
Index of malaria
25
20
15
10
5
0
LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (malaria only)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated

Figure 13. Interactions between Plasmodium yoelii and Leishmania mexicana in C/57 mice. P. yoelii followed by L. mexicana in twelve weeks (+/- SE).


Index of leishmaniasis
10
8
6
4
2
0
-2
LEGEND
Leishmania (Leishmania only)
Leishmania (dual infection)
Malaria (dual infection)
a - Malaria inoculated
b - Leishmania inoculated
28
32
36
40
44
Time (weeks)
Index of malaria
25
20
15
10
5
0
a
b
0
4
16
20
24

Discussion

Previous experiments examined interactions between P. yoelii and L. mexicana in BALB/c mice. BALB/c mice infected with L. mexicana developed fulminating lesions which did not heal. Infection with P. yoelii resulted in these mice becoming even more susceptible to L. mexicana. Under certain circumstances the presence of L. mexicana also resulted in more severe malaria compared to mice infected only with P. yoelii.

In humans, L. mexicana normally produces a single lesion which heals spontaneously (Perez et al. 1978). Infection produced by L. mexicana in BALB/c mice therefore does not provide a comparable model of human disease. C/57 mice infected with L. mexicana develop lesions which initially ulcerate but subsequently heal. These benign lesions resemble those normally produced in humans by L. mexicana (Perez et al. 1979).

Results from the present study support previous conclusions based on interactions between P. yoelii and L. mexicana in BALB/c mice. Plasmodium yoelii parasitemia was markedly enhanced whenever L. mexicana was inoculated prior to the malaria (Figs. 9, 10, & 12). In BALB/c mice, significant mortality occurred during dual infections, whereas no deaths occurred in C/57 mice. Maximum disease enhancement occurred when the interval between inoculation of P. yoelii and L. mexicana was short (Figs. 9 & 10). As in BALB/c mice, increased P. yoelii parasitemia occurred prior to the development of clinically evident leishmaniasis (Fig. 9).

Surprisingly, inoculation of L. mexicana followed by P. yoelii in 12 weeks produced little enhancement of either the malaria or the leishmaniasis (Fig. 12). These results support those in BALB/c mice. While we have not examined immunological function during dual infections, lesion size decreased after week 8. This suggests that the host immune system was actively responding to L. mexicana by 12 weeks post-inoculation. Any effect produced by P. yoelii at this point might not influence the progression of L. mexicana in the mouse. It is also possible that establishment of L. mexicana within the host is affected by P. yoelii. Once established, the course of leishmaniasis might progress independently of concomitant parasites.

In regions of the world where malaria and leishmaniasis coincide geographically, interactions between these parasites may play an important role in determining disease outcome. Our results clearly demonstrate that not only is the clinical infection important in determining the course of concomitant parasitic infections, but that prior patent, or subpatent infections have the ability to affect disease outcome as well. Recognition and treatment of subpatent infections therefore might be of considerable significance to the success of primary treatment.

Chapter IV

METASTASIS OF *LEISHMANIA MEXICANA AMAZONENSIS* IN *LEISHMANIA* RESISTANT C/57 MICE FOLLOWING CONCOMITANT INFECTION WITH *PLASMODIUM YOELII*

Introduction

American cutaneous leishmaniasis (ACL) results from infection with *Leishmania mexicana* or *L. braziliensis* (Perez 1983). ACL exhibits a wide spectrum of clinical symptoms. In most instances the infection consists of a spontaneously healing ulcerative lesion (Barral et al. 1983). However, non-ulcerative lesions which eventually disseminate may occur (Perez et al. 1978). It is believed that the clinical manifestations of ACL reflect properties of the parasite involved as well as genetic background and immunological competence of the host (Hill 1986, Pearson et al. 1983).

Several murine models have been developed which mimic the diverse symptoms of ACL. Inbred C/57 mice normally develop localized lesions which spontaneously heal, similar to the most common forms of ACL in humans. On the other end of the spectrum, BALB/c mice exhibit symptoms that are characteristic of disseminated ACL (Perez et al. 1979). These strain differences purportedly reflect differences in the genetic susceptibility of the host. We report here on the

development of disseminated leishmaniasis in a Leishmania resistant strain of mouse (C/57) following a non-lethal malaria infection.

Materials and Methods

Animals

C/57 mice were obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts.

Malaria

Procedures for inoculating and quantifying P. yoelii have been described (Chapter 2).

Leishmaniasis

The Walter Reed strain 227 of Leishmania mexicana amazonensis was used to infect mice. Procedures for inoculating this parasite have been previously described (Chapter 2). After inoculation of L. mexicana, the diameter of the infected footpad and the contra-lateral uninfected footpad (control) were measured weekly with a direct reading vernier caliper.

Experimental design

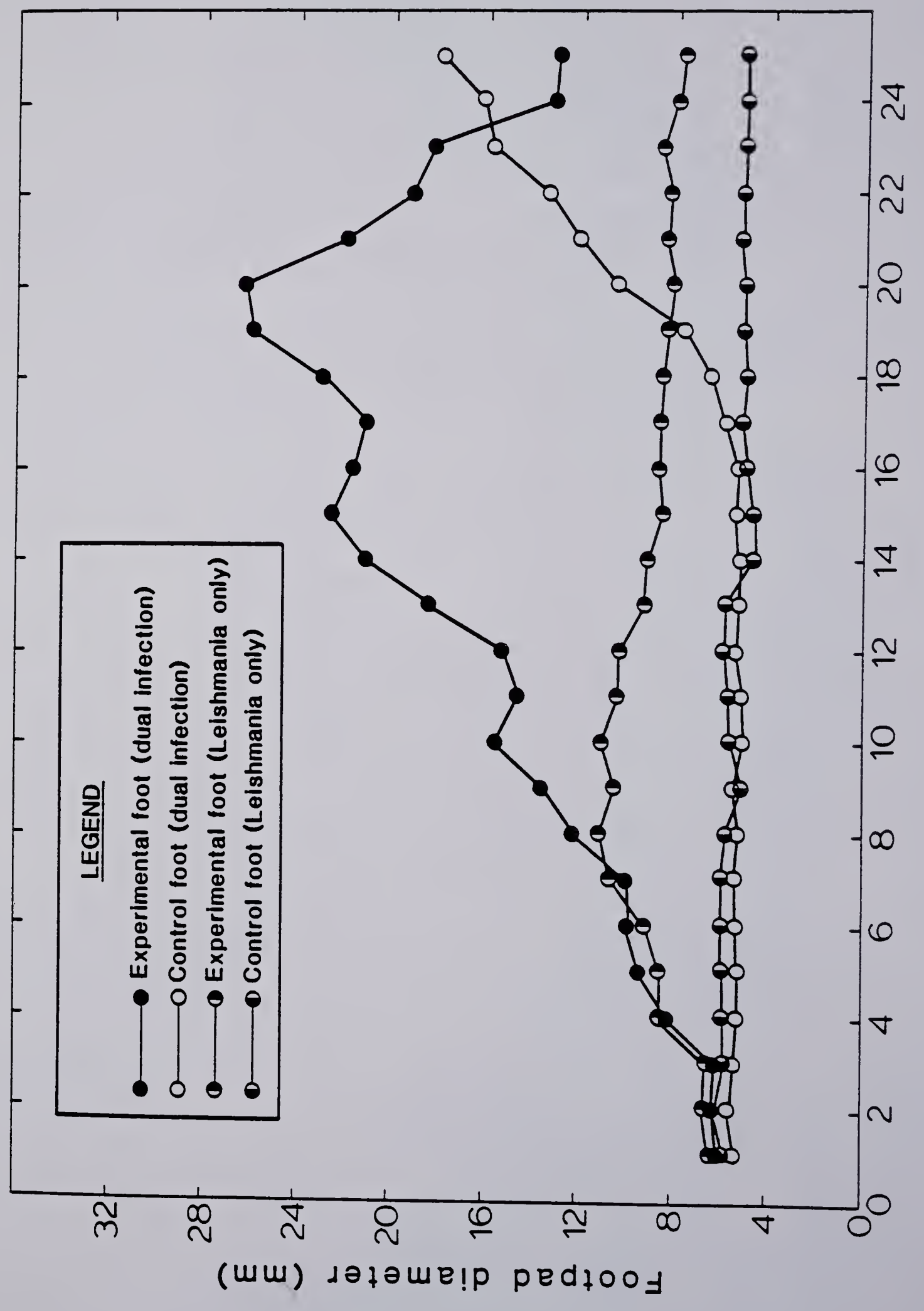
Twenty mice were inoculated with L. mexicana only. Ten additional mice were inoculated intra-peritoneally with L. mexicana followed by 1 x 10^6 Plasmodium yoelii infected RBC in 3 weeks. Development of L. mexicana was quantified for each group of mice.

Results

Lesion development in mice infected only with L. mexicana is shown in Figure 14. Lesion diameter peaked at about 8 weeks post-inoculation, with gradual healing of the foot thereafter. No dissemination to the contra-lateral footpad occurred. This observation has been confirmed in numerous other experiments. We have followed the course of infection of L. mexicana in approximately 150 C/57 mice for periods ranging from 1 - 1 1/2 years. In none of these mice did any visible dissemination of the parasites occur.

Footpad lesion size was greatly enhanced in those mice infected with both L. mexicana and P. yoelii. Mean maximum lesion size in these mice peaked at 14-16 mm in diameter, compared to a 10 mm diameter in mice infected only with L. mexicana. Three of the mice infected with both P. yoelii and L. mexicana developed lesions which reached a maximum diameter of 27 mm at 20 weeks post-inoculation. Atrophy of the foot thereafter resulted in a decrease in measurable lesion diameter, but did not indicate healing had occurred. All 3 of these mice developed lesions in the contra-lateral uninfected footpad at around 18 weeks post-inoculation (Figure 14). Metastatic lesions were also evident in the nasal and tail regions of 1 of these mice.

Figure 14. Footpad Diameter in mice infected with only Leishmania mexicana (N = 20), and in 3 mice infected with both Leishmania mexicana and Plasmodium yoelii that developed disseminated lesions. L. mexicana was inoculated at week 0 and P. yoelii at week 3.


LEGEND
Experimental foot (dual infection)
Control foot (dual infection)
Experimental foot (Leishmania only)
Control foot (Leishmania only)
Footpad diameter (mm)
0
4
8
12
16
20
24
28
32
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (weeks)

Discussion

Why some individuals develop disseminated ACL is not known. Barrel et al. (1983) found that a given parasite strain could cause both diffuse cutaneous leishmaniasis and muco-cutaneous leishmaniasis. They suggested that the clinical expression of the parasite was determined by the host response. Dissemination of the pathogen occurred only after mice were over a year old. Progressive depression of cell-mediated immunity, as pointed out by Gardner (1980), was suggested as possibly contributing to disease manifestation at this point. Dissemination of L. mexicana in this study occurred when mice were only 26 - 35 weeks old, suggesting that deterioration of the immune system did not play a role in parasite dissemination. Failure of immune mechanisms has also been suggested as a factor that could lead to development of disseminated ACL (Hill 1986). Walton et al. (1973) believed that pulmonary tuberculosis, alone or combined with severe malnutrition, could possibly breach immune defenses. This would precipitate invasion of mucosal tissue by L. braziliensis after years of latent infection.

Malaria is a well documented suppressor of immune function (Lelchuk & Playfair 1980, Lwin et al. 1982), and numerous studies have shown that malaria can enhance the course of concomitant infections (Cook 1985, Krettli 1977, Salaman et al. 1969). Our results suggest that concurrent infection with malaria has the potential to change a normally trivial Leishmania infection into one with serious consequences.

Chapter V

EFFECT OF CIMETIDINE, RANITIDINE, AND 2'-DEOXYGUANOSINE ON THE DEVELOPMENT OF *LEISHMANIA MEXICANA AMAZONENSIS* IN *LEISHMANIA* SUSCEPTIBLE BALB/C MICE AND IN *LEISHMANIA* RESISTANT C/57 MICE

Introduction

Leishmaniasis is a disease infecting millions of people in tropical and semitropical regions (Marinkelle 1980). Infection in vertebrate hosts is initiated through inoculation of flagellated promastigote forms by the sand fly vector. Promastigotes are taken up by mononucleated phagocytic cells and differentiate into amastigotes, which function as obligate intracellular parasites (Molyneux & Ashford 1983).

Pentavalent antimonials are the drugs most often used to treat leishmaniasis. Other compounds may be used when parasites respond poorly to these agents (Rees et al. 1985). Currently available chemotherapeutic agents have a number of drawbacks, including significant numbers of treatment failures (Bryceson et al. 1985, Thakur et al. 1984), occasional unexplained deaths (Rees et al. 1985), and potentially debilitating side effects (Peters et al. 1980, Thakur 1986). New, safer and cheaper antileishmanial agents are urgently needed (Marsden et al. 1979, Peters et al. 1980).

Host defense against Leishmania is believed to be modulated by cell-mediated immunity (Murray et al. 1986). Recovery from infection has been shown to be mediated by T cells in inbred strains of mice. Production of macrophage-activating lymphokines by spleen cells is important to the development of resistance (Coutinho et al. 1984, Scott & Sher 1986). Leishmania susceptible (e.g., BALB/c) mice characteristically exhibit a suppression of cell-mediated immune responses (Liew et al. 1982, Murray et al. 1986). In these non-cure mice, suppressor T cells may initiate anergy (Blackwell & Ulczak 1984, Nickol & Bonventre 1985a 1985b).

Modulation of immune response offers a potential means of controlling leishmaniasis. Monoclonal antibodies against specific T cell subsets have been used therapeutically to treat susceptible mice infected with leishmaniasis (Titus et al. 1985), and specific transfer of T cell populations has been shown to enhance delayed hypersensitivity responses to L. major (Lima et al. 1984). A number of agents are currently used to control various human diseases by modulating immune response (Bender et al. 1984, Bril et al. 1984, Drews 1985, Osband et al. 1981). Cimetidine is an H-2 histamine receptor antagonist which can inhibit T suppressor cell function (Zapata Sirvent et al. 1985). It also can enhance proliferative responses of peripheral blood lymphocytes (Gifford et al. 1980). Cimetidine has been used successfully to control peptic ulcers (Brogden et al. 1978) and tumors (Osband et al. 1981), and can restore suppressed immunity following burns (Bender et al. 1984). Ranitidine is also a H_2 histamine receptor antagonist, but the central imidazole group found in

cimetidine has been replaced with a furan ring, and a side chain has been slightly modified (Henry et al. 1980). The result is a compound which inhibits the binding of histamine to H_2 receptors yet has little affinity for other sites (Zeldis et al. 1983). T suppressor cell activity is also affected by 2'-deoxyguanosine. This agent acts by inhibiting the enzyme ribonucleotide reductase, thereby preventing DNA synthesis in these cells (Bril et al. 1984).

The purpose of this study was to determine if cimetidine, ranitidine, and 2'-deoxyguanosine could be used to control L. mexicana amazonensis infection in BALB/c mice. Sodium stibogluconate (pentostam) is a widely used antileishmanial drug. This agent was therefore used as a baseline drug against which to compare the efficacy of these immuno-modulating agents.

Materials and Methods

Animals

BALB/c and C/57 mice were obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts. Eight-week-old females weighing 25-30 g were used for all experiments.

Leishmaniasis

Walter Reed strain 227 of Leishmania mexicana amazonensis was used. The source and history of this isolate have been described (Nolan et al. 1984), as have methods for the purification and inoculation of parasites (Chapter 2). Amastigotes were obtained from donor mice. The donor was sacrificed and the infected foot

amputated, surface sterilized with 70% ETOH, and homogenized in a Ten-Broeck glass tissue grinder. Amastigote viability was determined by the method of Hodgkinson and Herman (1980) and the number of parasites per unit volume calculated by using a Petroff-Housser bacterial cell counting chamber. The solution was adjusted with PBS to obtain a final concentration of 10^2, 10^3, 10^4, 10^5, or 10^6 viable amastigotes in 10 ul PBS. Mice were infected by subcutaneously inoculating 10 ul of the appropriate solution into the ventral surface of the right rear-footpad. The thickness of the infected footpad was measured weekly with a direct-reading vernier caliper. Measurements commenced 1 week prior to the inoculation of L. mexicana and continued for 10 (BALB/c) or 20 (C/57) weeks. Lesion diameter was expressed as the thickness of the infected footpad in millimeters minus the thickness of the contralateral uninfected footpad.

Drug treatment

Drug treatment commenced 1 day after inoculation of L. mexicana into mice, and was continued once daily for either 10 or 20 days. Thereafter, mice were inoculated once a week until the conclusion of the experiment. Inocula were delivered in 0.5 ml PBS. Control mice received only PBS. Experimental groups consisted of 10 (BALB/c mice) or 20 (C/57 mice) animals.

Experimental groups

Experiment i. The BALB/c mice in this experiment received either cimetidine (20 mg/kg/day) or pentostam (200 mg/kg/day) once daily for 10 days.

Experiment ii. The BALB/c mice in this experiment received either cimetidine (40 mg/kg/day), pentostam (200 mg/kg/day), or 2'-deoxyguanosine (40 mg/kg/day) once daily over a 20 day period.

Experiment iii. BALB/c mice in this experiment were inoculated with combinations of cimetidine (40 mg/kg/day) and pentostam (200 mg/kg/day), cimetidine (40 mg/kg/day) and 2'-deoxyguanosine (40 mg/kg/day), or pentostam (200 mg/kg/day) and 2'-deoxyguanosine (40 mg/kg/day). The drugs were given once daily over a 20 day period.

Experiment iv. Eight groups of BALB/c mice were infected with 10^6 L. mexicana amastigotes in this experiment. Mice in each group were treated with cimetidine or ranitidine in one of the following doses: 40, 80, 160, or 320 mg/kg/mouse/day. A control group received PBS.

Experiment v. Ten groups of BALB/c mice were used in this experiment. Two groups of mice were each infected with 10^2, 10^3, 10^4, 10^5, or 10^6 viable L. mexicana amastigotes. One group of mice infected with each inocula received either cimetidine or ranitidine at a dose of 80 mg/kg/mouse/day. Control mice for each treatment received only PBS.

Experiment vi. In this experiment, two groups of C57 mice were infected with 10^6 L. mexicana amastigotes and treated with either

cimetidine or ranitidine (80 mg/kg/mouse/day). A control group received PBS.

Results

Experiment i

Results from the first experiment are shown in Fig. 15. Lesions in mice which had received cimetidine or pentostam were consistantly smaller than the lesions in control mice. The amount of variation in lesion size within each group of mice was initally small. However, the degree of variation increased markedly between 6 and 9 weeks post-inoculation in those mice treated with cimetidine.

Experiment ii

Increasing the dose of cimetidine or 2'-deoxyguanosine and/or the duration of treatment resulted in the development of smaller lesions than in control animals. Cimetidine produced the most marked therapeutic effect, whereas pentostam and 2'-deoxyguanosine resulted in less impressive control (Fig. 16).

Experiment iii

All combinations of drugs were more effective at controlling lesion development than were therapeutic regimes in which either pentostam or 2'-deoxyguanosine was used alone (Fig. 17). However, none of the drug combinations which were tested were more effective at controlling L. mexicana infections than cimetidine (Figs. 16 and 17).

Experiment iv

Results from the fourth experiment are shown in Figs. 18 and 19. Increasing the doses of both cimetidine (Fig. 18) and ranitidine (Fig. 19) from 40 mg/kg/mouse/day to 320 mg/kg/mouse/day resulted in a corresponding decrease in the diameter of lesions. No mortality was observed, except in that group of mice receiving ranitidine at a dose of 320 mg/kg/day. Seven of the eight mice in this group died within 2 h of the first drug treatment. Significant lesion reduction was observed as early as 4 weeks post-inoculation with both agents (Figs. 18 & 19). Complete resolution resulting from drug treatment was not noted in any instance.

Experiment v

When the infective inocula of L. mexicana was increased a corresponding increase in lesion diameter was observed (Figs. 20-24). Neither cimetidine (80 mg/kg/day) nor ranitidine (80 mg/kg/day) had a marked effect on lesion development in those mice infected with only 10^2 or 10^3 amastigotes (Figs. 20 & 21). Cimetidine was more effective than ranitidine when the infective dose consisted of 10^4 amastigotes (Fig. 22), while ranitidine was more efficient at limiting lesion development when mice were infected with 10^5 or 10^6 amastigotes (Figs. 23 & 24). No mortality occurred in any of these mice.

Experiment vi

The progression of L. mexicana amazonensis in BALB/c mice is markedly different that that normally seen in humans infected with

this pathogen. In this respect C/57 mice provide a more realistic model of human infection with the parasite. Both cimetidine and rantidine were effective at limiting the development of L. mexicana in these mice (Fig. 25). Lesions in both experimental groups were smaller throughout the course of the infection, and healing of lesions occurred more rapidly in treated mice than in control mice (Fig. 25).

Figure 15. Effect of cimetidine (20 mg/kg/day) and pentostam (200 mg/kg/day) on the development of Leishmania mexicana in BALB/c mice. Drugs were given for a 10 day period commencing 1 day after inoculation of L. mexicana (+/- SE).

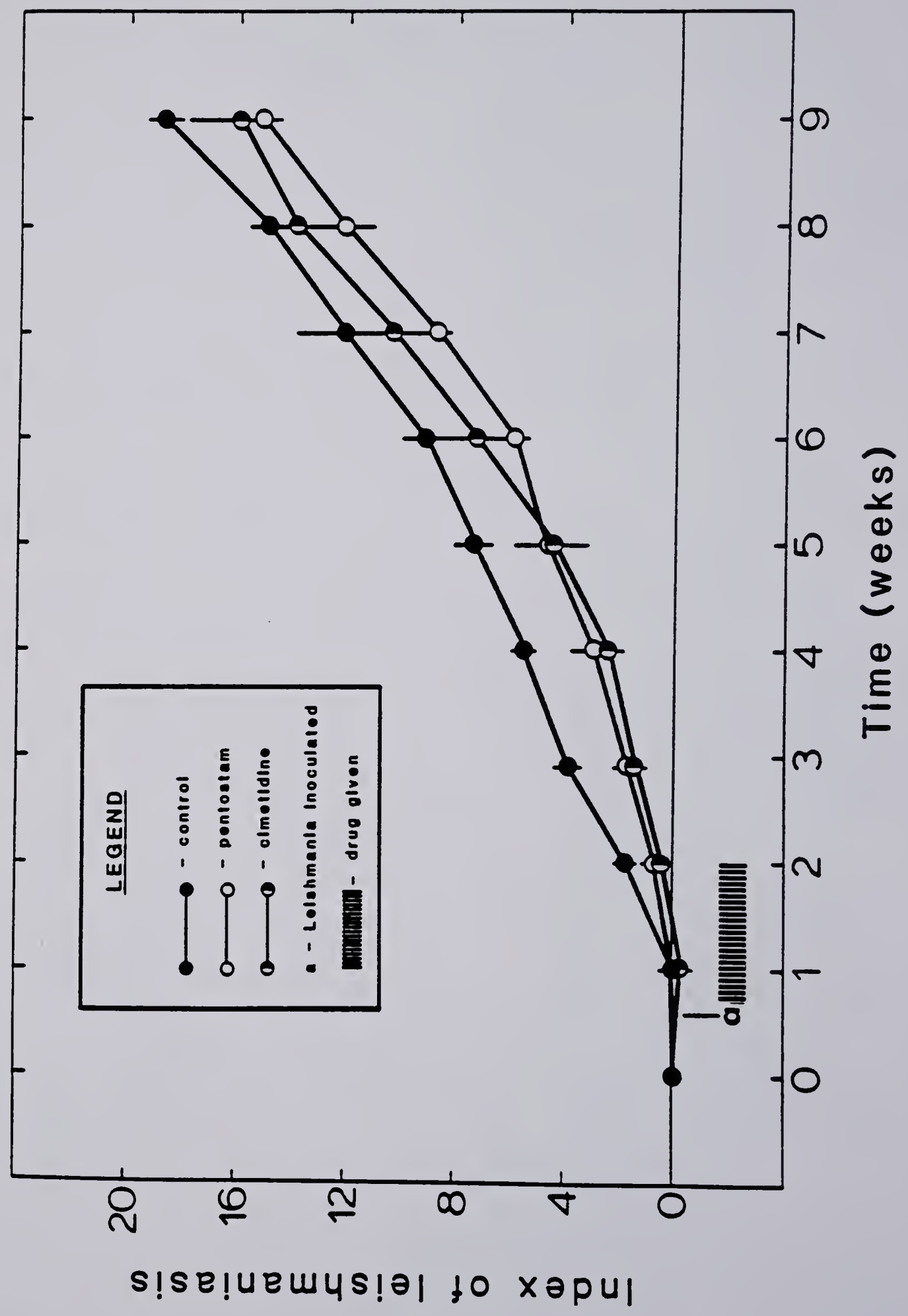

Index of leishmaniasis
20
16
12
8
4
0
0
1
2
3
4
5
6
7
8
9
Time (weeks)
a
LEGEND
- control
- pentostam
- cimetidine
a - Leishmania inoculated
- drug given

Figure 16. Effect of cimetidine (40 mg/kg/day), pentostam (200 mg/kg/day), and 2'-deoxyguanosine (40 mg/kg/day) on the development of Leishmania mexicana in BALB/c mice. Drugs were given for a 20 day period commencing 1 day after inoculation of L. mexicana (+/- SE).

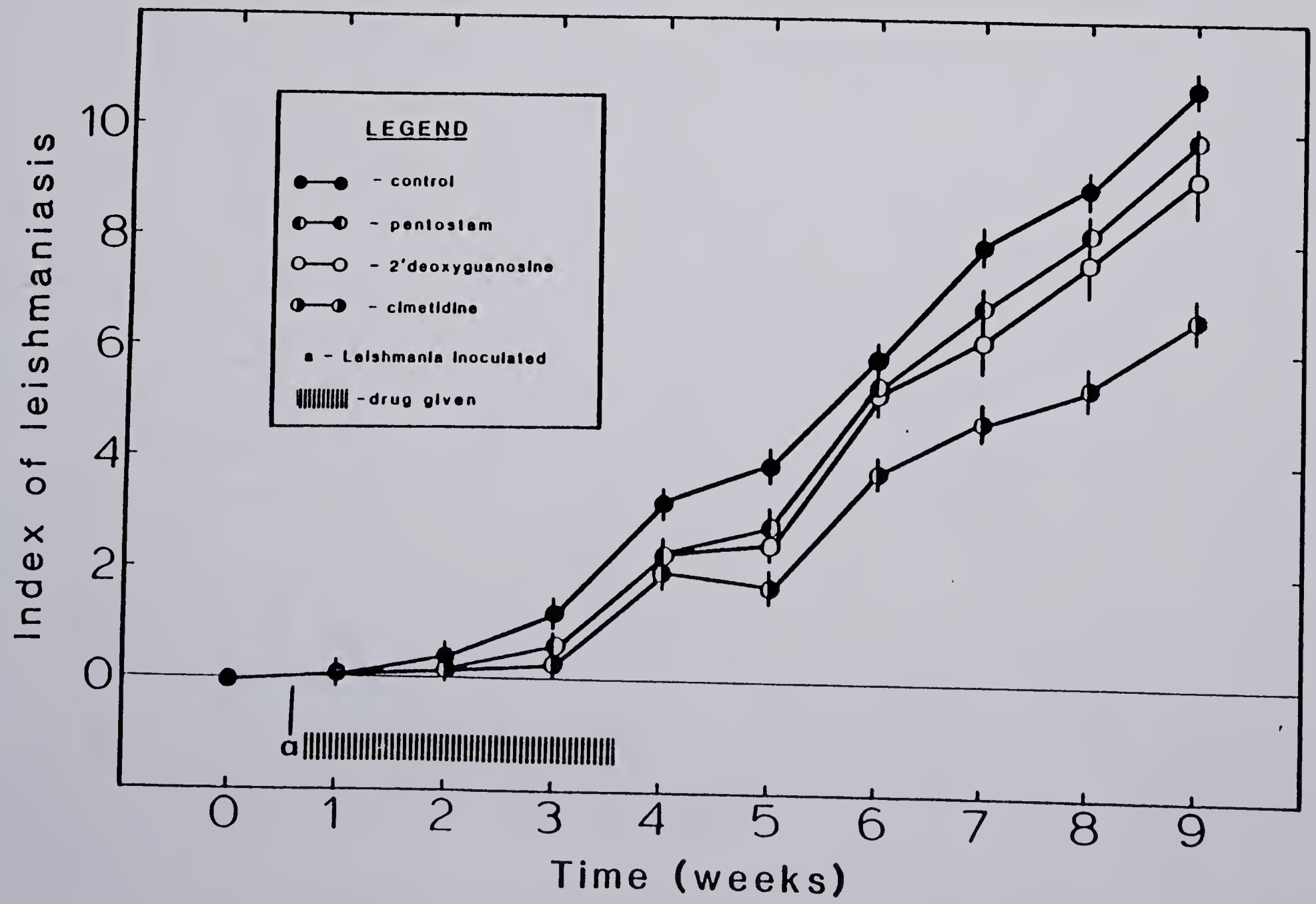

LEGEND
- control
- pentostam
- 2'deoxyguanosine
- cimetidine
a - Leishmania inoculated
- drug given
Index of leishmaniasis
0
2
4
6
8
10
a
0
1
2
3
4
5
6
7
8
9
Time (weeks)

Figure 17. Effect of cimetidine (40 mg/kg/day) and pentostam (200 mg/kg/day), 2'-deoxyguanosine (40 mg/kg/day) and pentostam (200 mg/kg/day), and cimetidine (40mg/kg/day) and 2'-deoxyguanosine (40 mg/kg/day), on the development of Leishmania mexicana in BALB/c mice. Drugs were given for a 20 day period commencing 1 day after inoculation of L. mexicana (+/- SE).

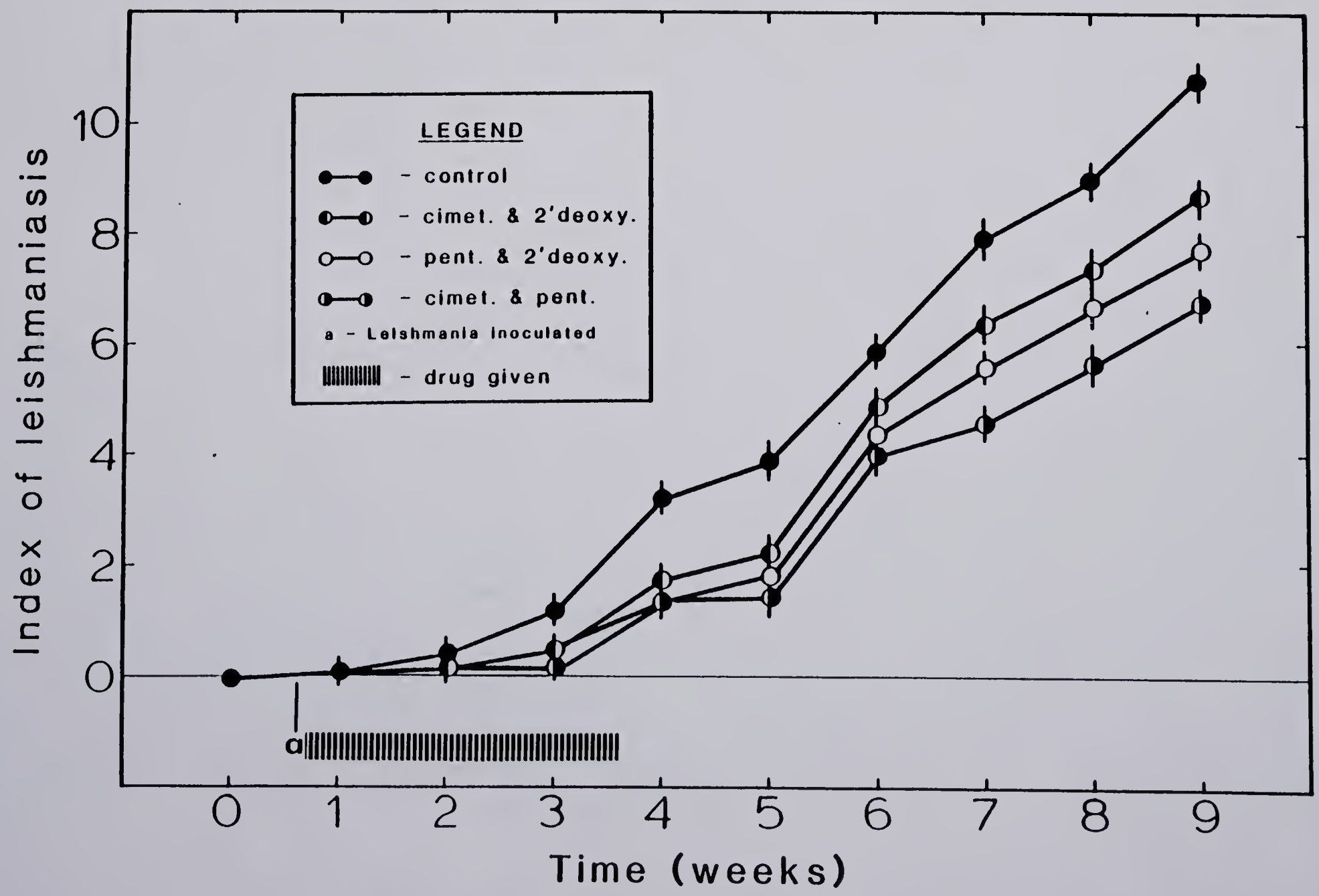

LEGEND
- control
- cimet. & 2'deoxy.
- pent. & 2'deoxy.
- cimet. & pent.
a - Leishmania inoculated
- drug given
Index of leishmaniasis
10
8
6
4
2
0
a
0 1 2 3 4 5 6 7 8 9
Time (weeks)

Figure 18. Effect of cimetidine (40, 80, 160, and 320 mg/kg/day) on the development of Leishmania mexicana amazonensis in BALB/c mice. Mice were infected with 10^6 amastigotes at Week 0 (+/- SE).

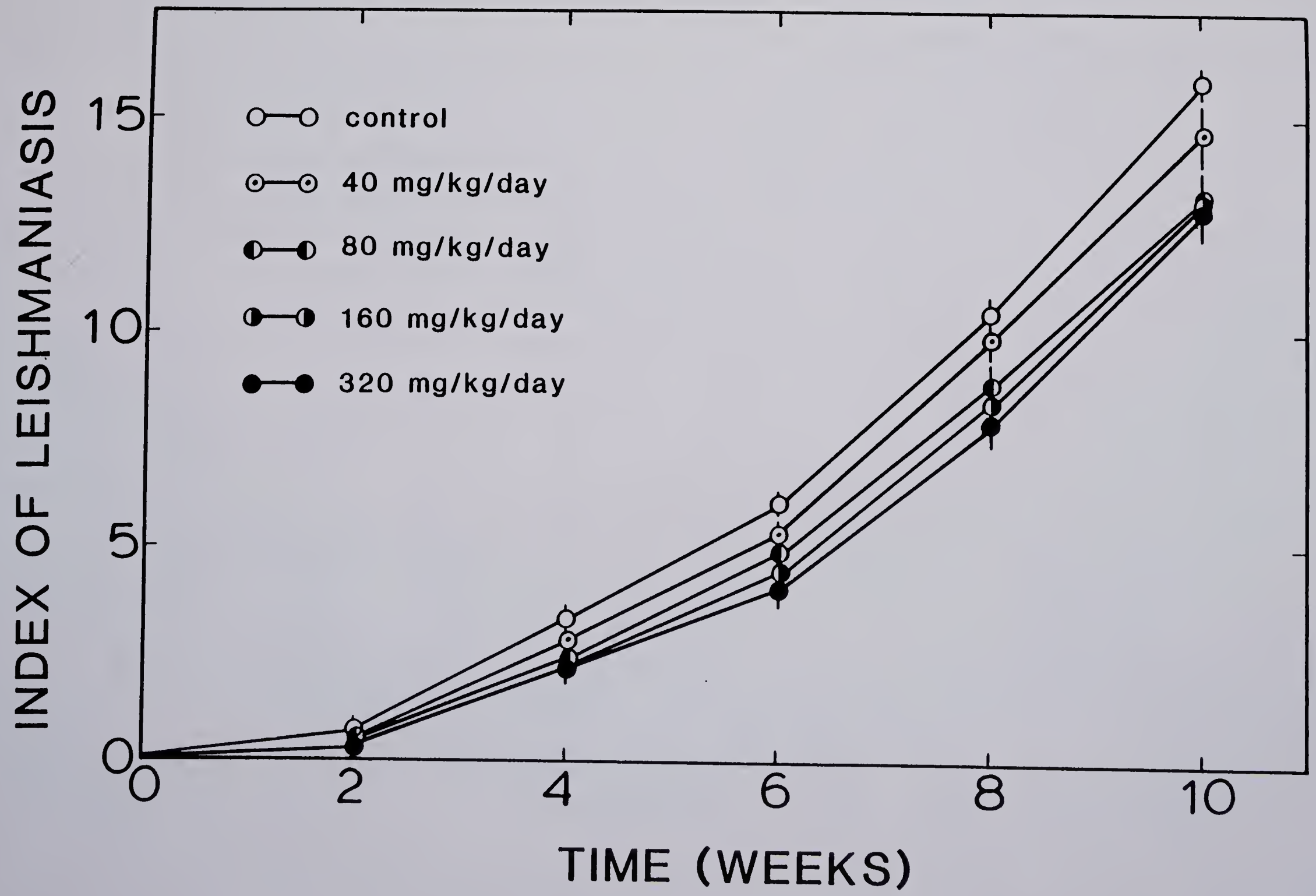

INDEX OF LEISHMANIASIS
15
10
5
0
0
2
4
6
8
10
TIME (WEEKS)
control
40 mg/kg/day
80 mg/kg/day
160 mg/kg/day
320 mg/kg/day

Figure 19. Effect of ranitidine (40, 80, 160, and 320 mg/kg/day) on the development of Leishmania mexicana amazonensis in BALB/c mice. Mice were infected with 10^6 amastigotes at Week 0 (+/- SE).

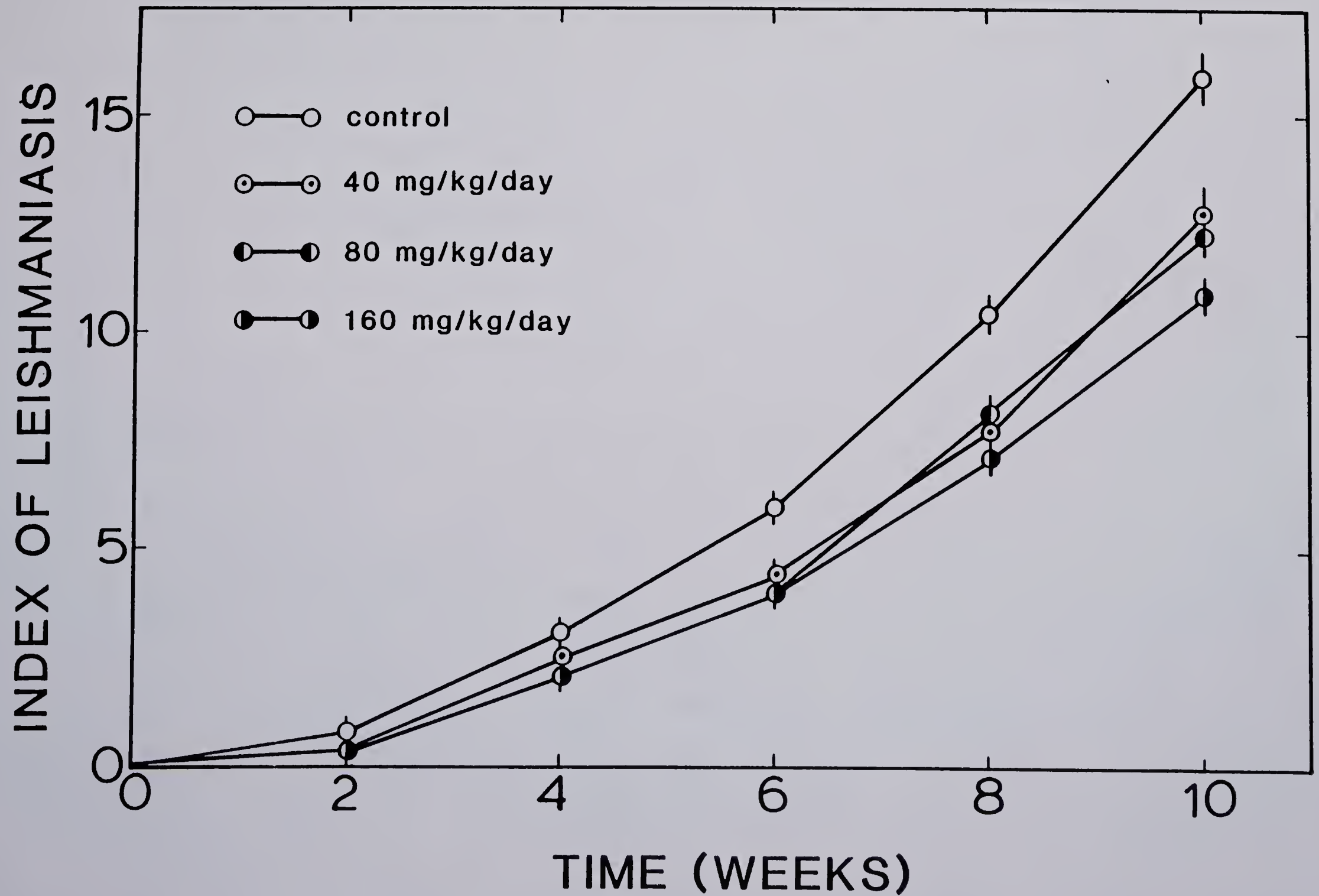

control
40 mg/kg/day
80 mg/kg/day
160 mg/kg/day
INDEX OF LEISHMANIASIS
0
5
10
15
0
2
4
6
8
10
TIME (WEEKS)

Figure 20. Effect of cimetidine and ranitidine (80 mg/kg/day) on the development of Leishmania mexicana amazonensis in BALB/c mice. Mice were infected with 10^2 viable amastigotes on Week 0 (+/- SE).

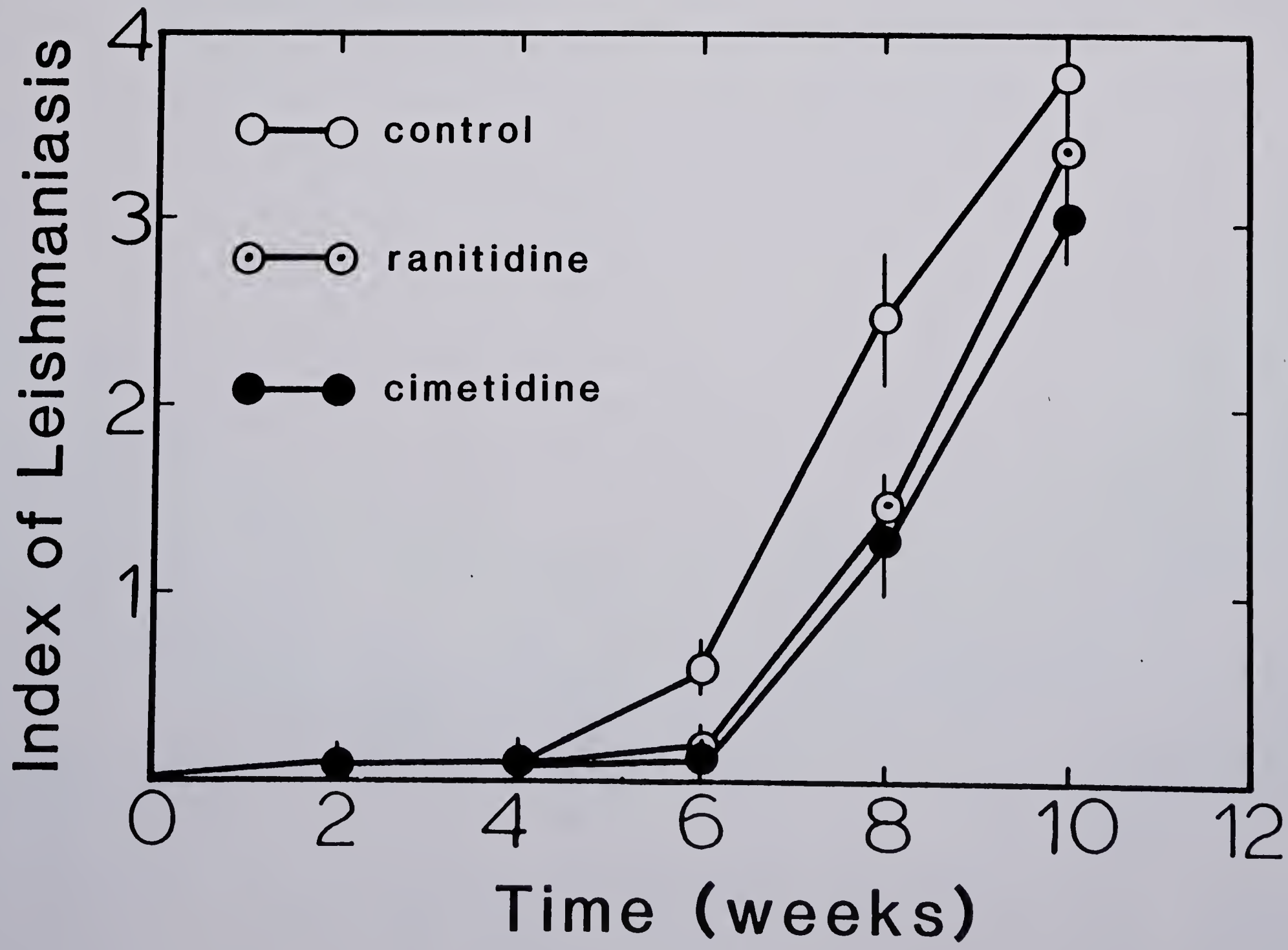

Index of Leishmaniasis
4
3
2
1
control
ranitidine
cimetidine
0
2
4
6
8
10
12
Time (weeks)

Figure 21. Effect of cimetidine and ranitidine (80 mg/kg/day) on the development of Leishmania mexicana amazonensis in BALB/c mice. Mice were infected with 10^3 viable amastigotes on Week 0 (+/- SE).

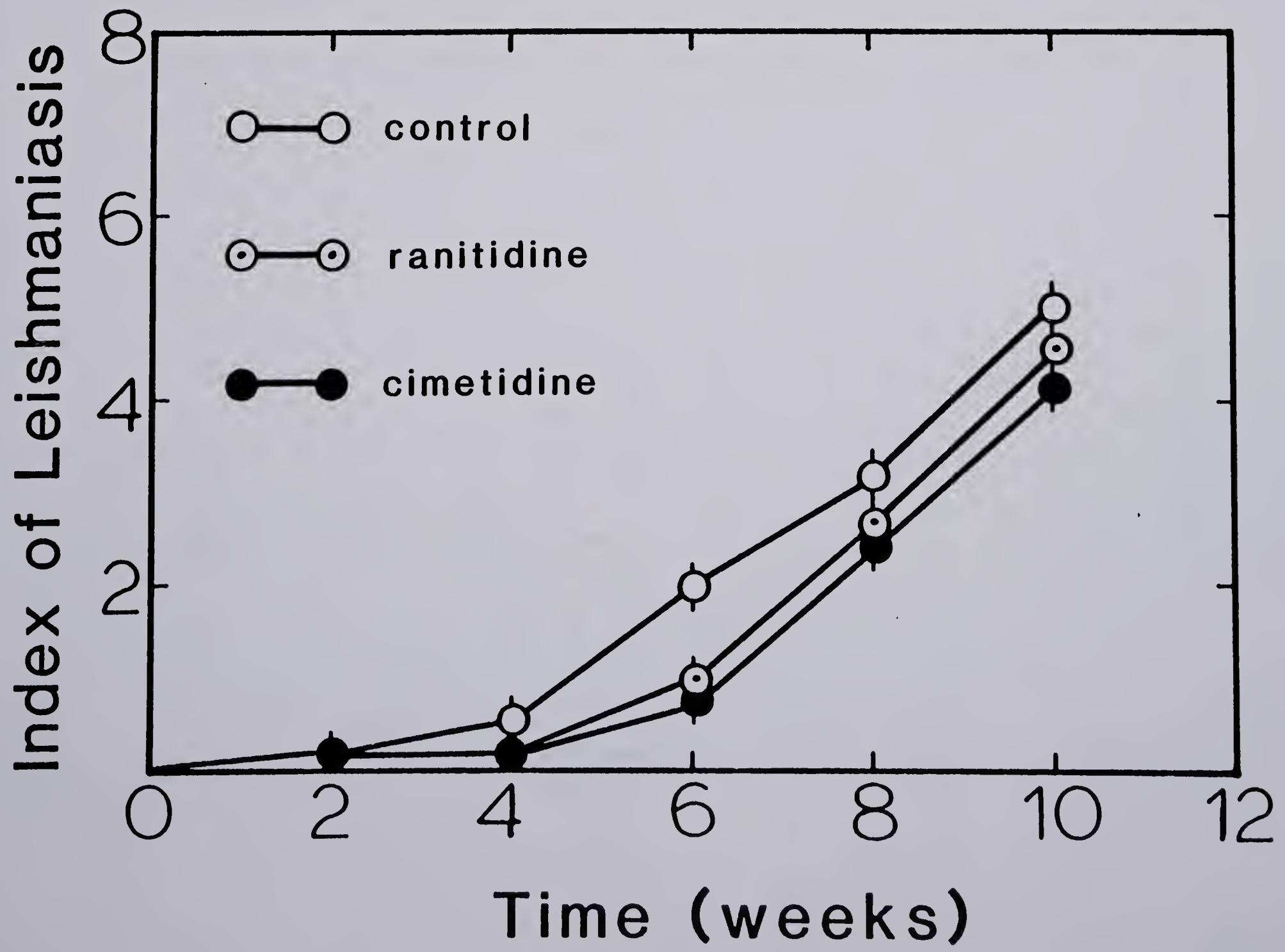

control
ranitidine
cimetidine
Index of Leishmaniasis
0
2
4
6
8
0
2
4
6
8
10
12
Time (weeks)

Figure 22. Effect of cimetidine and ranitidine (80 mg/kg/day) on the development of *Leishmania mexicana amazonensis* in BALB/c mice. Mice were infected with 10^4 viable amastigotes on Week 0 (+/- SE).

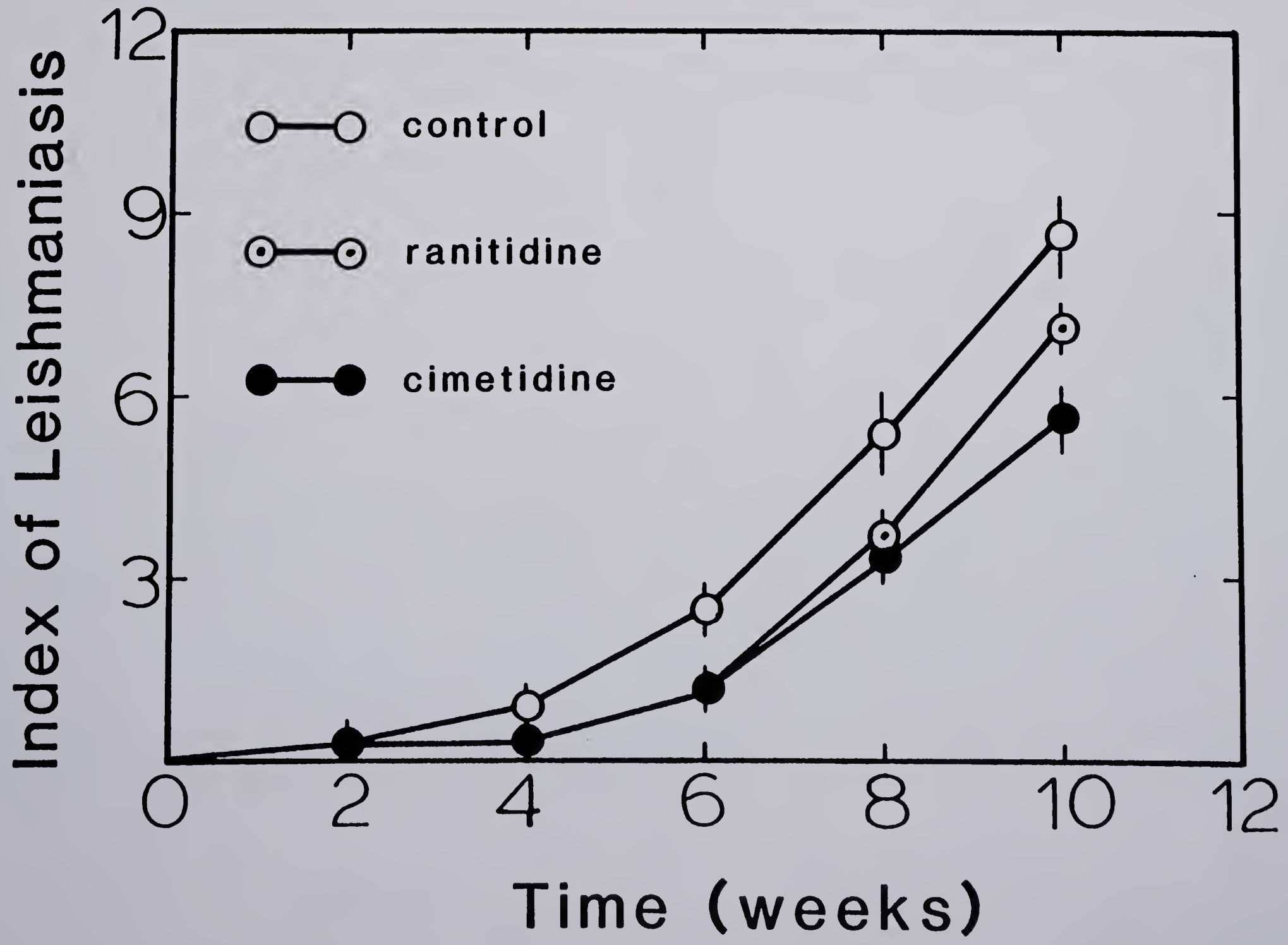

Index of Leishmaniasis
12
9
6
3
0
2
4
6
8
10
12
control
ranitidine
cimetidine
Time (weeks)

Figure 23. Effect of cimetidine and ranitidine (80 mg/kg/day) on the development of Leishmania mexicana amazonensis in BALB/c mice. Mice were infected with 10^5 viable amastigotes on Week 0 (+/- SE).

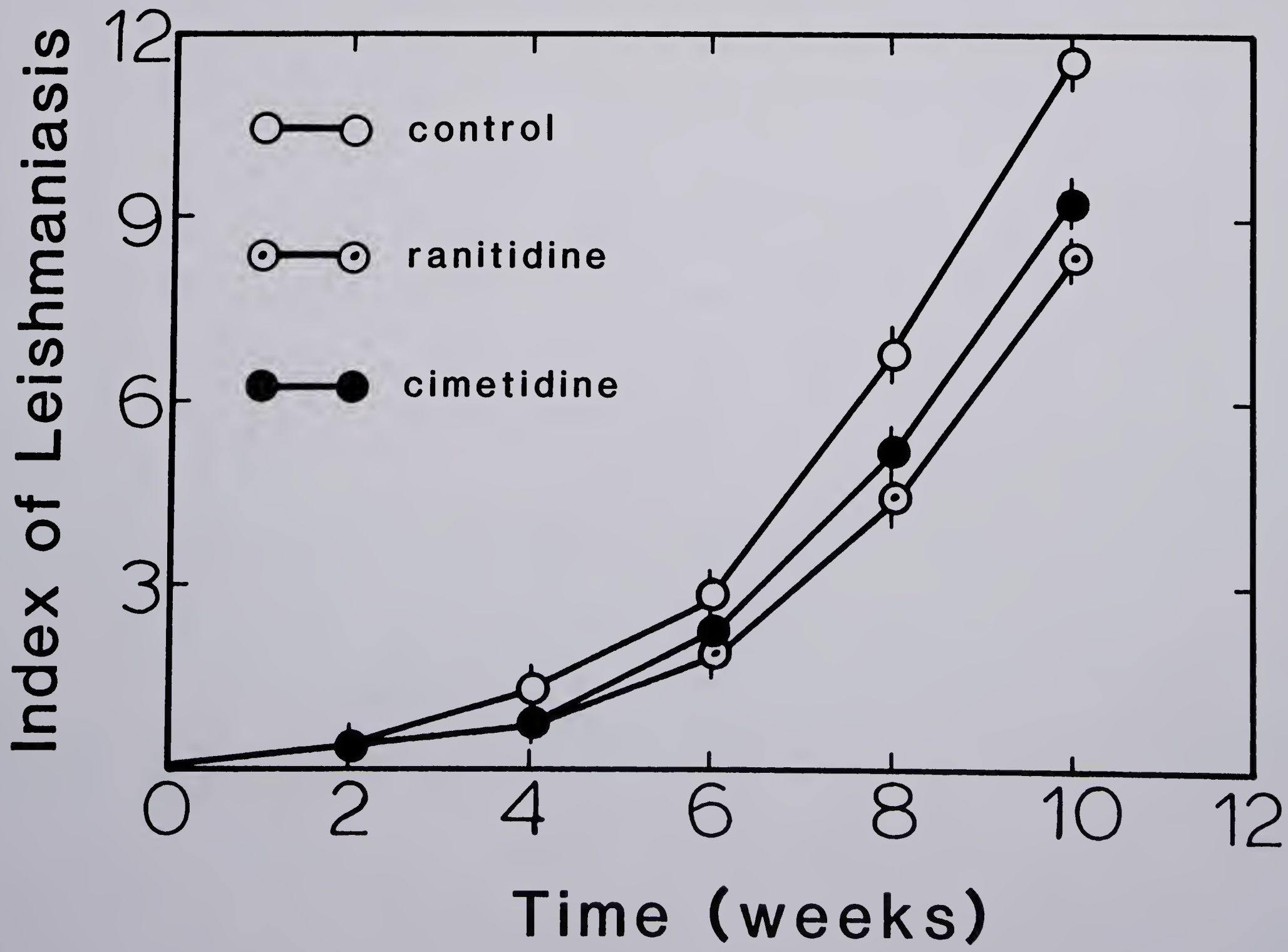

Index of Leishmaniasis
12
9
6
3
0
2
4
6
8
10
12
control
ranitidine
cimetidine
Time (weeks)

Figure 24. Effect of cimetidine and ranitidine (80 mg/kg/day) on the development of Leishmania mexicana amazonensis in BALB/c mice. Mice were infected with 10^6 viable amastigotes on Week 0 (+/- SE).

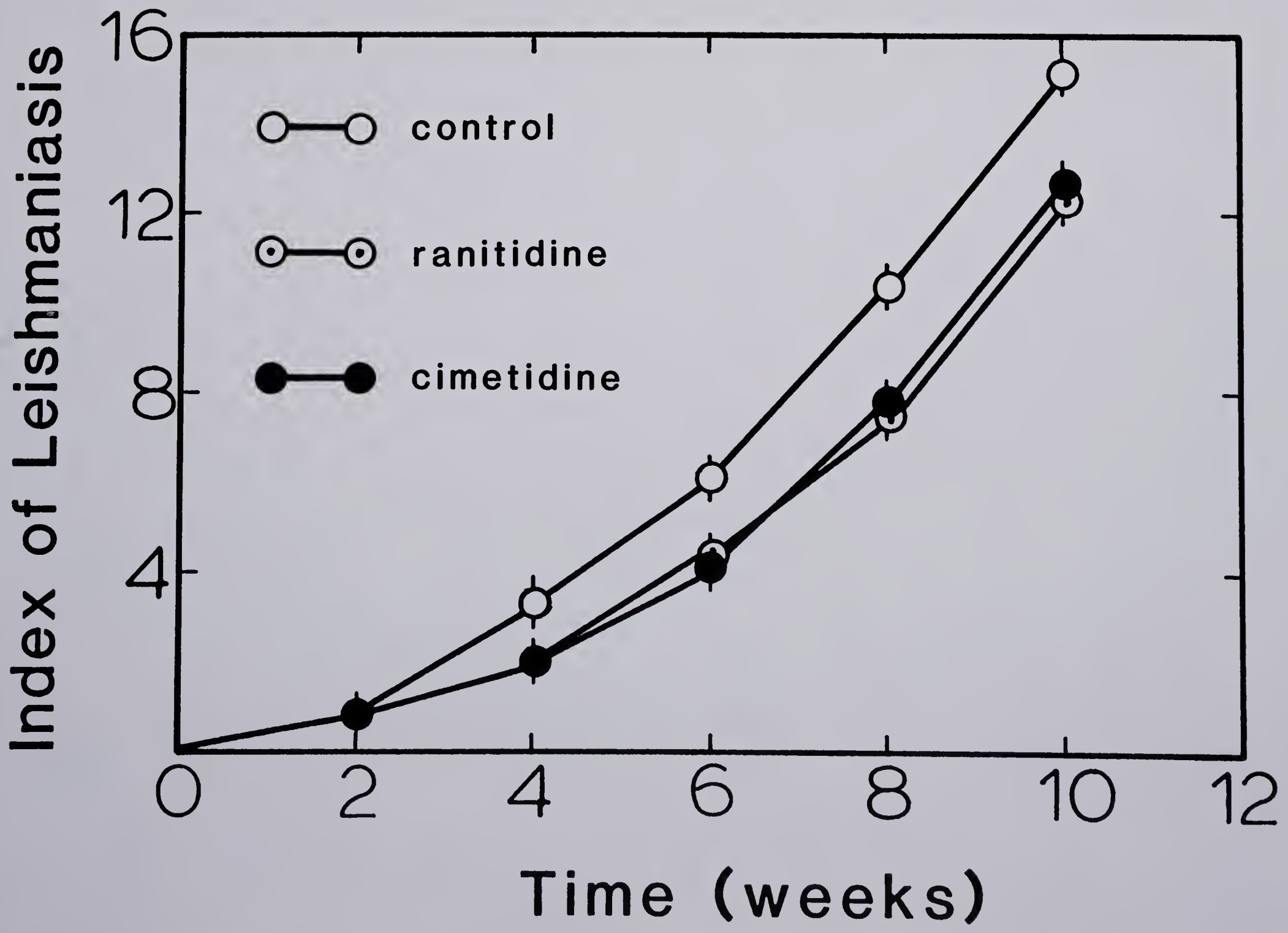
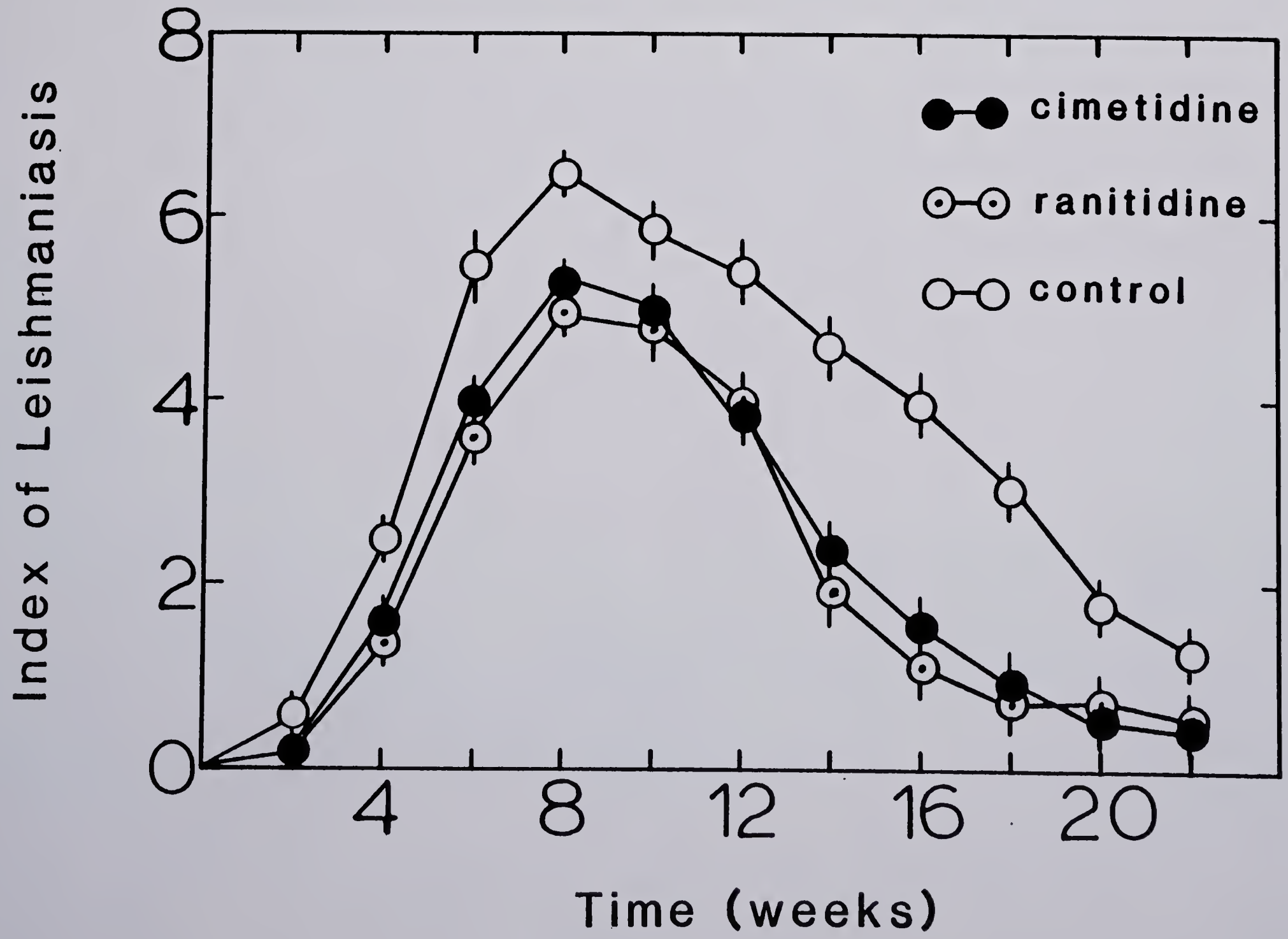

Index of Leishmaniasis
16
12
8
4
control
ranitidine
cimetidine
0
2
4
6
8
10
12
Time (weeks)

Figure 25. Effect of cimetidine and ranitidine (80 mg/kg/day) on the development of Leishmania mexicana amazonensis in C/57 mice. Mice were infected with 10^6 viable amastigotes on Week 0 (+/- SE).


Index of Leishmaniasis
0
2
4
6
8
4
8
12
16
20
Time (weeks)
cimetidine
ranitidine
control

Discussion

Mice infected with cutaneous leishmaniasis can be divided into "healers" and "non-healers." Specific immune responses control the infection in "healers", whereas in susceptible mice suppressor T cells abrogate potentially curative cell-mediated immunity (Howard et al. 1980). These results suggest that specific immunotherapy aimed at T suppressor cells can provide control superior to that obtained with pentostam in BALB/c mice.

Cimetidine, an agent that inhibits T suppressor cell function, can evidently affect the development of L. mexicana amazonensis in BALB/c and C/57 mice. This control persisted through the course of the infection. In most instances where the immuno-restorative effect of cimetidine has been examined, the development of a particular disease was slowed rather than completely resolved (Osband et al. 1981, Gifford et al. 1981). In no instance, in Leishmania susceptible or resistant mice, did cimetidine completely eliminate the development of clinical symptoms of cutaneous leishmaniasis, supporting these previous data.

Cimetidine affects the function of all cells bearing the H-2 histamine receptor (Zapata-Sirvent et al. 1985). Ranitidine also competitively inhibits the binding of histamine to H_2-receptors; however, unlike cimetidine, ranitidine purportedly does not bind to other sites such as androgen receptors, the hepatic mixed-function oxidase system, or peripheral lymphocytes (Zeldis et al. 1983). As such, ranitidine should not effect immune function. Although we have

not examined the immunological response of ranitidine treated mice during L. mexicana infections, ranitidine was as effective as cimetidine at limiting the development of clinical symptoms, suggesting that immunological cells might be affected by this agent.

2'-deoxyguanosine was less effective than cimetidine at controlling lesion development, but more effective than pentostam. 2'-deoxyguanosine inhibits replication of T suppressor cells, but does not affect the activity of functional cells (Bril et al. 1984), while cimetidine affects the function of all cells bearing the H-2 histamine receptor (Zapata-Sirvent et al. 1985). Reduction of T suppressor cell populations is presumably more complete with cimetidine than with 2'-deoxyguanosine, possibly explaining the limited effect of 2'-deoxyguanosine on development of lesions.

The BALB/c mouse provides a suitable model to study diffuse cutaneous leishmaniasis (DCL); however, the rapid development and progression of lesions followed by fatal visceralization is atypical of the majority of human infections (Perez et al. 1979). None of the agents we used to treat BALB/c mice were completely effective in limiting development of the parasite (Figs. 15-17). In spite of this, BALB/c mice proved ideal as an experimental host in which to compare the efficacy of various agents at reducing the severity of Leishmania derived lesions. Differences in rates of lesion development resulting from various drug regimes were easily quantified and significant differences were routinely demonstrated. Use of a mouse strain which is somewhat more resistant to L. mexicana could provide additional evidence on the beneficial effects of immuno-modulating agents.

The effect of the infective dose towards parasite development is often overlooked, as is the role the infective dose has on the efficacy of therapeutic agents. Ranitidine and cimetidine were examined under a variety of situations in this study. Both cimetidine and ranitidine were most effective when extraordinary inocula were used to infect mice. When lower, presumably more realistic, doses of infective inocula were used (i.e., 10^2 or 10^3 parasites), the effect on lesion development was less dramatic.

Our results in both BALB/c and C/57 mice suggest that cimetidine and ranitidine have potential as antileishmanial agents. Unless an adequate immune response is mounted, muco-cutaneous and diffuse cutaneous leishmaniasis often respond poorly to currently used therapeutic regimes. Combining agents which affect immune function, such as cimetidine, with currently used anti-leishmanial agents might diminish the number of therapeutic failures experienced. The wealth of literature available on the chemistry, mode of action, and side effects of cimetidine increase the attractiveness of this agent (Brogden et al. 1978). Although ranitidine is not as widely used as cimetidine, a number of studies have indicated that the agent is a more potent H_2-receptor antagonist than cimetidine. The attractiveness of the agent is further augmented by the evident lack of complicating side-effects which may mitigate the efficacy of cimetidine. In light of these results, it is somewhat paradoxical that ranitidine purportedly does not affect immune function (Zeldis et al. 1983). Ranitidine clearly exhibited a curative effect on the development of leishmaniasis in mice; in most instances the drug was more effective

than cimetidine as an anti-leishmanial agent. However, our results do not indicate whether immune function was enhanced or whether the agent acted in some other fashion. In view of the limited number of agents which are currently used to treat human leishmaniasis, the anti-leishmanial potential of cimetidine, ranitidine, and 2'-deoxy-guanosine should be examined in further detail.

Chapter VI

THE EFFECT OF ANTI-LEISHMANIAL CHEMOTHERAPY ON THE DEVELOPMENT OF LEISHMANIASIS (*LEISHMANIA MEXICANA AMAZONENSIS*) AND CONCOMITANT MALARIA (*PLASMODIUM YOELII*) IN *LEISHMANIA* SUSCEPTIBLE BALB/C MICE

Introduction

Many of the currently used chemotherapeutic agents inhibit immune function, both *in vivo* and *in vitro* (Thong & Ferrante 1978, Thong et al. 1978 1979, Hanson 1981). Many protozoan diseases, including malaria and leishmaniasis, have well documented immunosuppressive capabilities (Bruce-Chwatt 1985, Killick-Kendrick & Peters 1978, Molyneux & Ashford 1983). The therapeutic use of agents which compromise the immune system would only serve to exacerbate the problem caused by parasite with known immunosuppressive function (Ferrante & Goh 1984). Accordingly, drugs used in the treatment of specific diseases could be selected on the basis of their secondary effects on immune function, not just on their antiparasitic activity (Hanson 1981).

We have previously shown that concurrent infection with *Leishmania mexicana amazonensis* can influence the development of *Plasmodium yoelii* in BALB/c and C/57 mice (Chapters 2, 3, and 4).

Although we have no direct evidence of this, disease enhancement which resulted from concomitant infection with these pathogens may be due to the well documented immunosuppressive capabilities of these parasitic protozoa (Killick-Kendrick & Peters 1978, Liew et al. 1982, Murray et al. 1986). We have also demonstrated that agents which modulate immune function can effectively be used to treat L. mexicana infections in BALB/c and C/57 mice. 2'-deoxyguanosine, cimetidine, and ranitidine were as effective at limiting the development of localized cutaneous lesions in these mice as pentostam, a widely used anti-leishmanial agent (Chapters 5 & 6).

This study was designed to assess the impact of pentostam and cimetidine on the development of P. yoelii in BALB/c mice. Plasmodium yoelii infections were initiated in uninfected mice and in mice which had been previously infected with L. mexicana.

Materials and Methods

Animals

Female BALB/c mice were obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts. Mice were eight weeks old and weighed 25-30 g when experiments were initiated.

Leishmaniasis

Walter Reed strain 227 of Leishmania mexicana amazonensis was used. The source and history of this isolate have been described (Nolan et al. 1984), as have methods for the purification and inoculation of parasites (Chapter 2). Mice were infected with 1×10^6

amastigotes obtained from donor mice which had been infected 8 weeks prior to the initiation of this experiment. Progress of the infection was determined on a weekly basis by measuring the diameter of both rear feet with a direct-reading vernier caliper. The size of the lesion (in mm) was calculated by subtracting the measurement obtained for the uninfected foot from that of the contralateral infected foot. Measurements commenced 1 week prior to the inoculation of L. mexicana and continued for 10 weeks.

Malaria

Procedures for the inoculation of experimental animals and the quantification of Plasmodium yoelii parasitemia have been described (Chapter 2). All mice were infected with 1×10^6 infected erythrocytes.

Drug treatment

Pentostam (200 mg/kg/day) and cimetidine (80 mg/kg/day) were used. Drug treatment commenced 1 day after the inoculation of parasites and was continued once daily for 20 days. Mice were thereafter inoculated once a week until the conclusion of the experiment. Inocula were delivered i.p. in 0.5 ml phosphate-buffered saline (PBS), with control mice only receiving PBS.

Experimental groups

Two experiments were conducted. Mice were treated with pentostam in the first experiment and cimetidine in the second. Four groups of 20 mice were used in each experiment. These groups

consisted of: 1) uninfected mice, 2) P. yoelii infected mice, 3) L. mexicana infected mice, and 4) mice infected with both P. yoelii and L. mexicana (L. mexicana inoculated 1 day prior to P. yoelii). Ten of the mice in each group were treated with the appropriate therapeutic agent. The 10 remaining mice were treated with PBS and served as controls.

Results

The effect of pentostam on parasite development is shown in Figures 26-28. Pentostam limited the development of cutaneous lesions in mice which were only infected with L. mexicana and in mice which were concurrently infected with P. yoelii (Fig. 26). As previously demonstrated, treatment with pentostam did not "cure" any of the mice which were infected with L. mexicana; however, the progression of the disease was slowed in these animals.

Plasmodium yoelii infected mice that were treated with pentostam developed malaria which was somewhat more severe than that in untreated animals (Fig. 27). The infection in these treated mice peaked 10 days after pathogen inoculation, when 20% of all erythrocytes were parasitized. Parasites were cleared from the peripheral circulation by day 16 of the infection.

Mice which were concomitantly infected with both P. yoelii and L. mexicana developed more severe malaria than did those mice which were only infected with P. yoelii (Figs. 27 & 28). Up to 30 % of all

RBC were infected ten days after parasite inoculation, and the infection was not cleared until 18 days post-inoculation.

Dually infected mice which were treated with pentostam developed less severe P. yoelii infections than did dually infected animals which were untreated. However, the malaria in these treated mice was more severe than that which developed in mice which were only infected with P. yoelii (Figs. 27 & 28).

In Figure 28 the cumulative percent parasitemia in each group of mice is plotted over time. Differences in the course of parasite development that are not clear when plotted in a normal fashion are evident using this format. All animals which were treated with pentostam developed more severe malaria than untreated P. yoelii infected mice.

Cimetidine limited the development of leishmaniasis in mice infected with L. mexicana and in mice infected with both L. mexicana and P. yoelii (Fig. 29). The level of control observed with this agent was comparable with that obtained using pentostam.

Plasmodium yoelii infected mice which were treated with cimetidine developed less severe malaria than untreated mice. Parasites were cleared from the peripheral circulation by 14 days post inoculation in these treated mice compared to 16 days in control animals (Figs. 30 & 31). Mice which were concurrently infected with both L. mexicana and P. yoelii developed significantly more severe malarial infections than mice which were only infected with P. yoelii (Figs. 30 & 31). When these dually infected mice were treated with cimetidine the severity of the Plasmodium infection decreased

significantly, both in terms of maximum observed parasitemia as well as in the overall duration of the infection (Fig. 30). By plotting the cumulative percent parasitemia over time it is possible to clearly visualize the effect cimetidine had on parasite development (Fig. 31).

Figure 26. Effect of pentostam on the development of Leishmania mexicana in i) mice infected with L. mexicana only, and ii) in mice infected with both P. yoelii and L. mexicana. Parasites were inoculated at Week 0 (+/- SE).

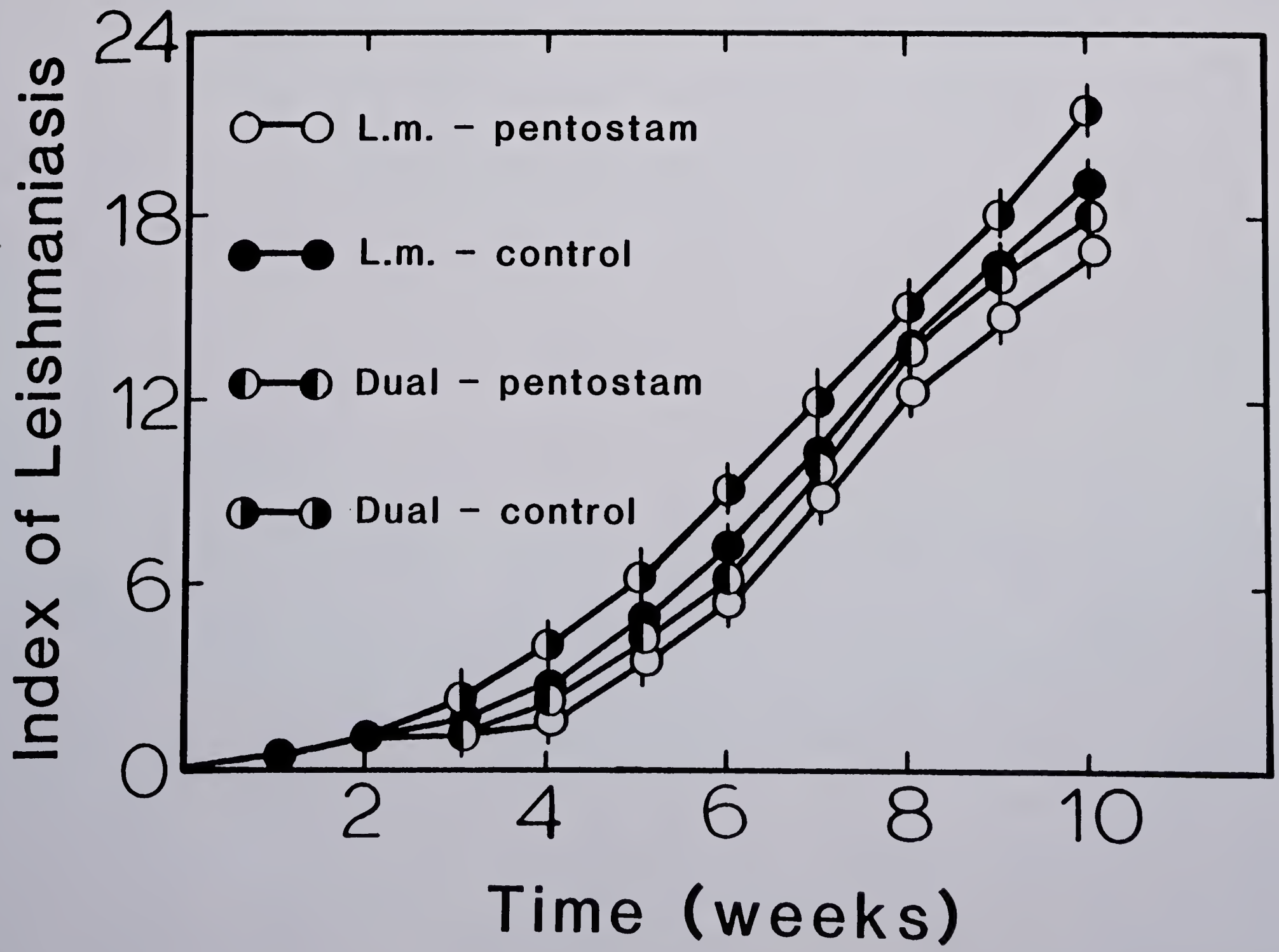

Index of Leishmaniasis
24
18
12
6
0
2
4
6
8
10
Time (weeks)
L.m. - pentostam
L.m. - control
Dual - pentostam
Dual - control

Figure 27. Effect of pentostam on the development of Plasmodium yoelii in mice infected with i) P. yoelii only, and ii) in mice infected with both P. yoelii and Leishmania mexicana. Parasites were inoculated at Day 0 (+/- SE).

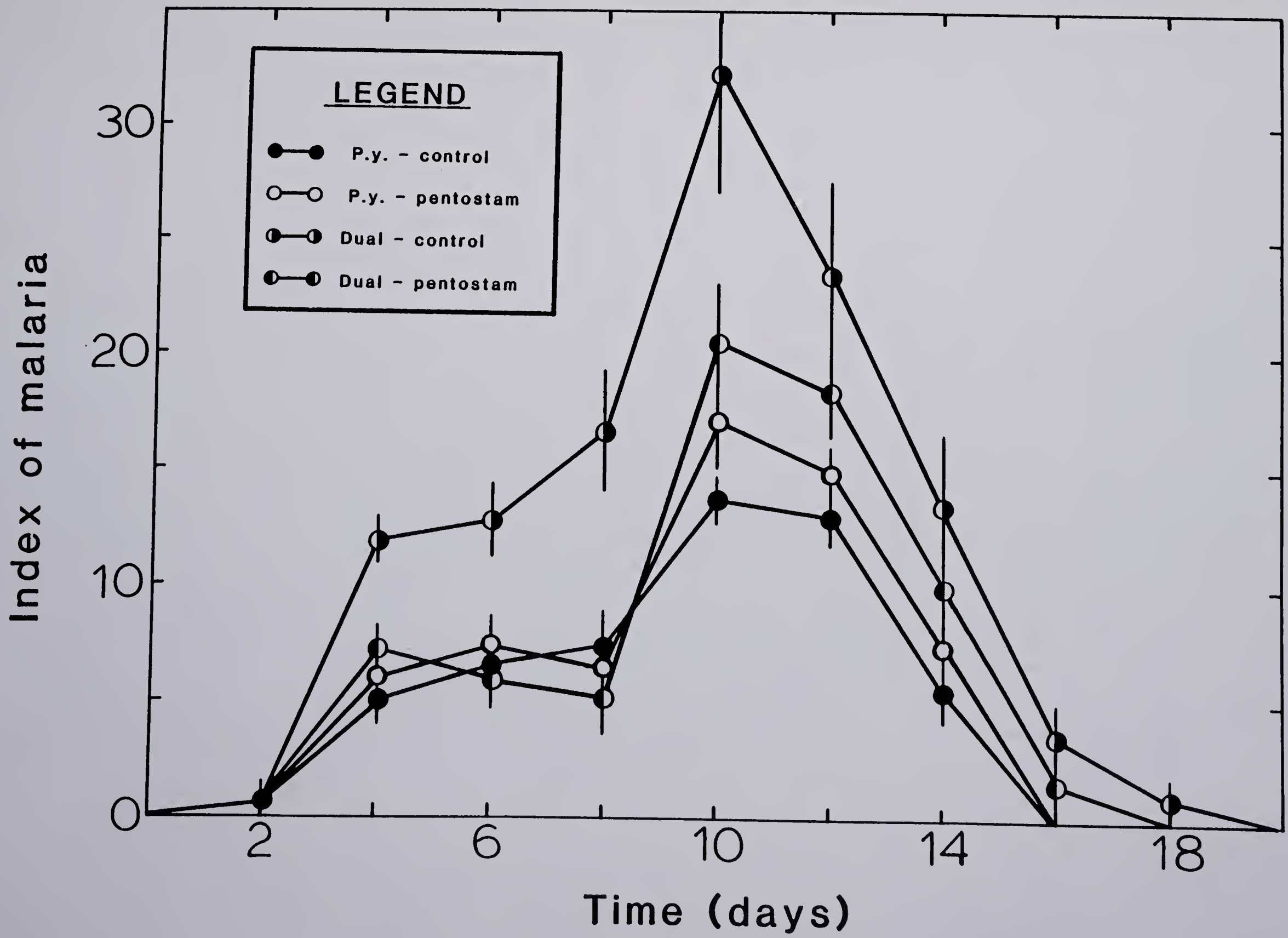

LEGEND
P.y. - control
P.y. - pentostam
Dual - control
Dual - pentostam
Index of malaria
0
10
20
30
2
6
10
14
18
Time (days)

Figure 28. Effect of pentostam on the development of Plasmodium yoelii in mice infected with P. yoelii only and in mice infected with both P. yoelii and Leishmania mexicana: Cumulative percent parasitemia as a means of differentiating the effects of the drug on malaria. Parasites were inoculated at Day 0.

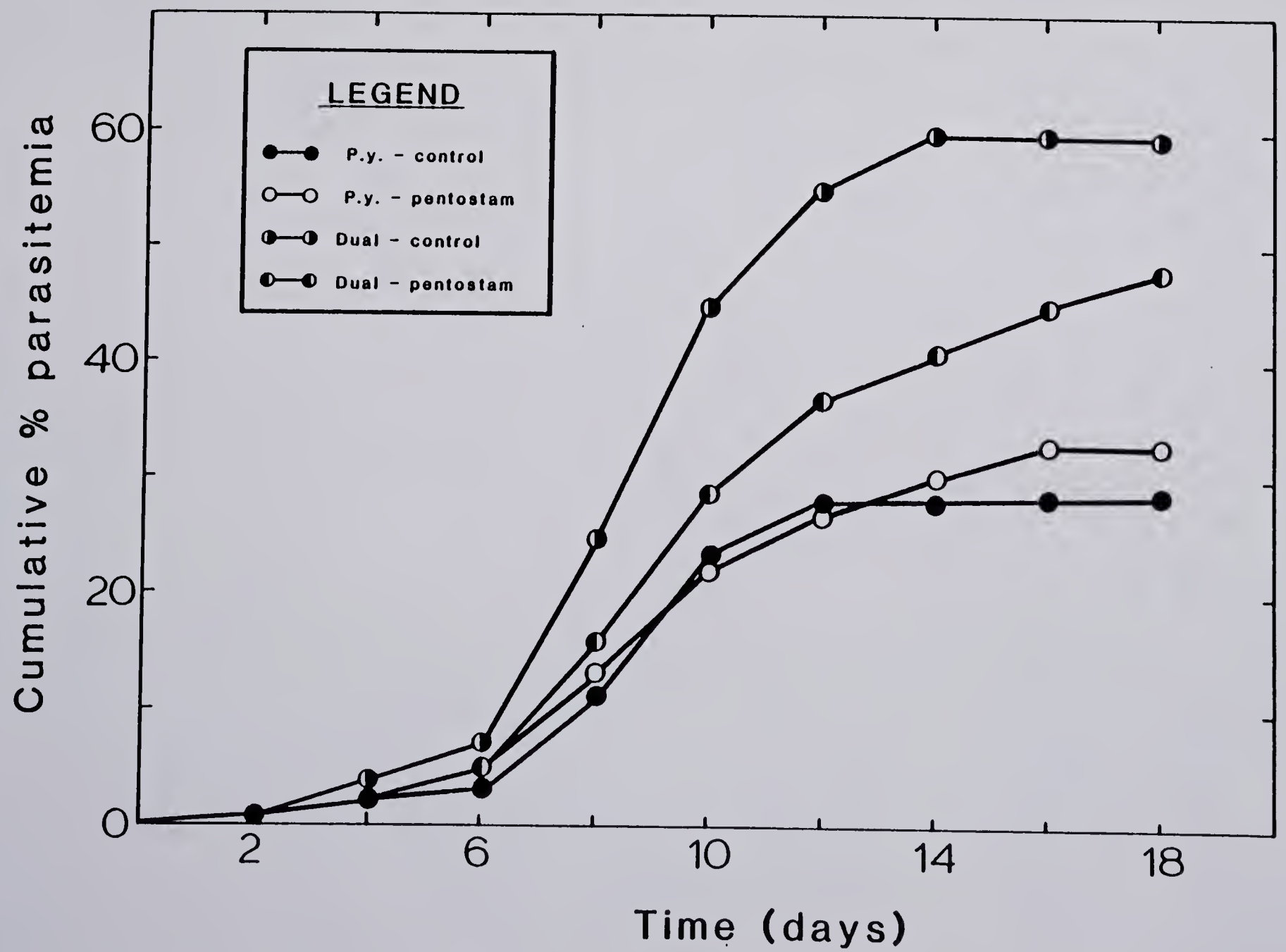

LEGEND
P.y. - control
P.y. - pentostam
Dual - control
Dual - pentostam
Cumulative % parasitemia
0
20
40
60
2
6
10
14
18
Time (days)

Figure 29. Effect of cimetidine on the development of Leishmania mexicana in i) mice only infected with L. mexicana, and ii) in mice infected with both Plasmodium yoelii and L. mexicana. Parasites were inoculated at Week 0 (+/- SE).

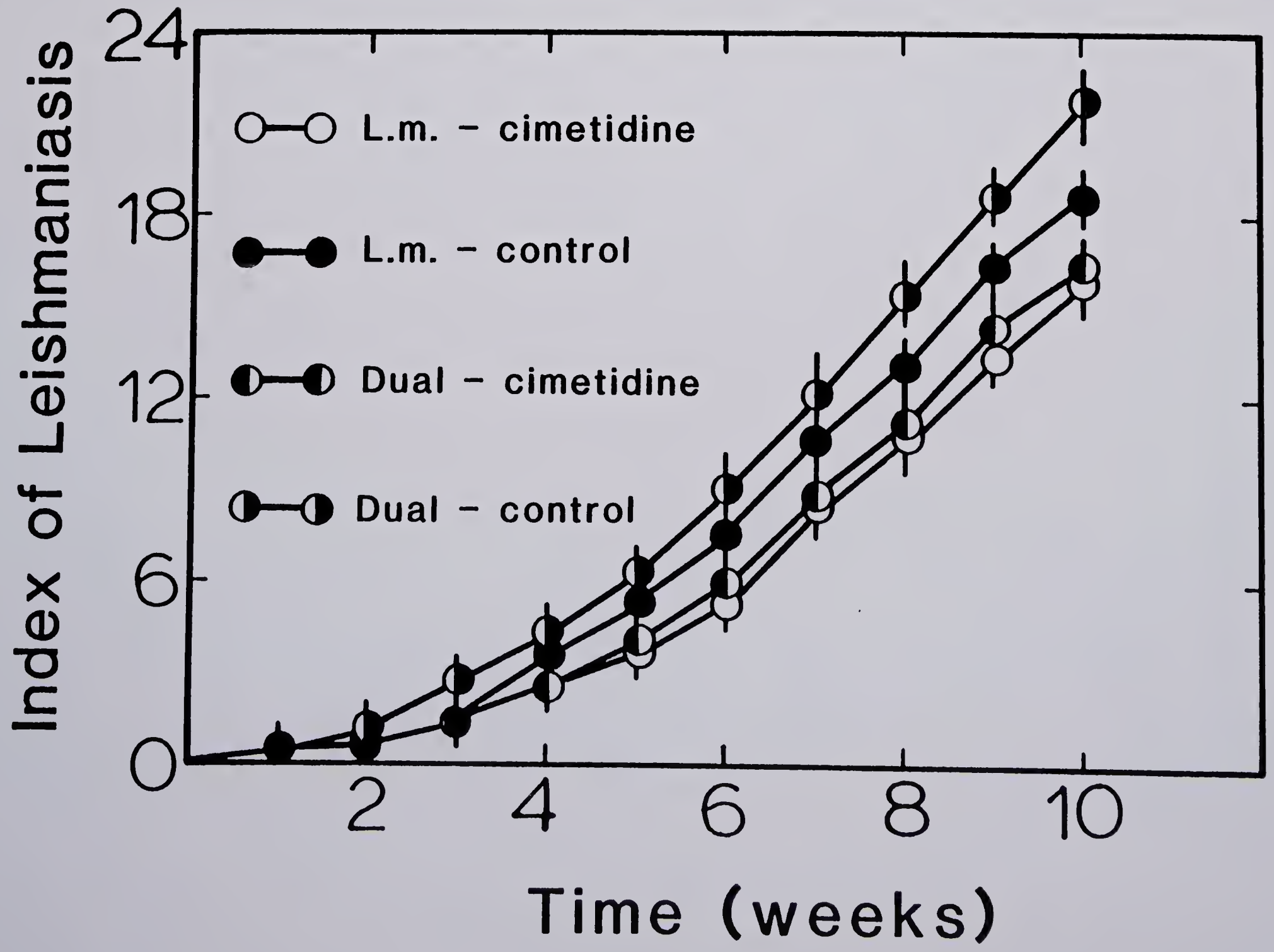

Index of Leishmaniasis
24
18
12
6
0
L.m. – cimetidine
L.m. – control
Dual – cimetidine
Dual – control
2
4
6
8
10
Time (weeks)

Figure 30. Effect of cimetidine on the development of Plasmodium yoelii in mice infected with i) P. yoelii only, and ii) in mice infected with both P. yoelii and Leishmania mexicana. Parasites were inoculated at Day 0 (+/- SE).

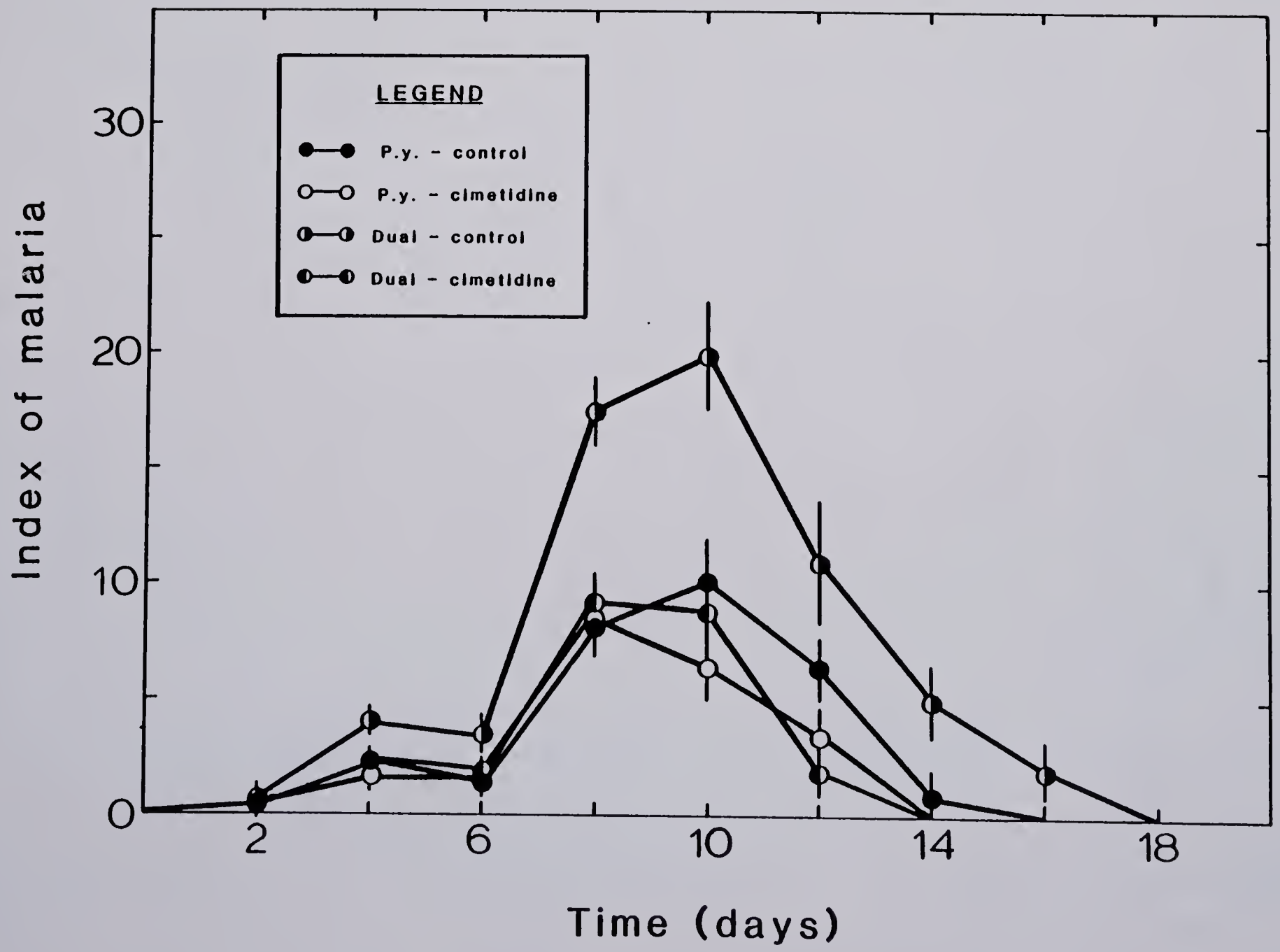
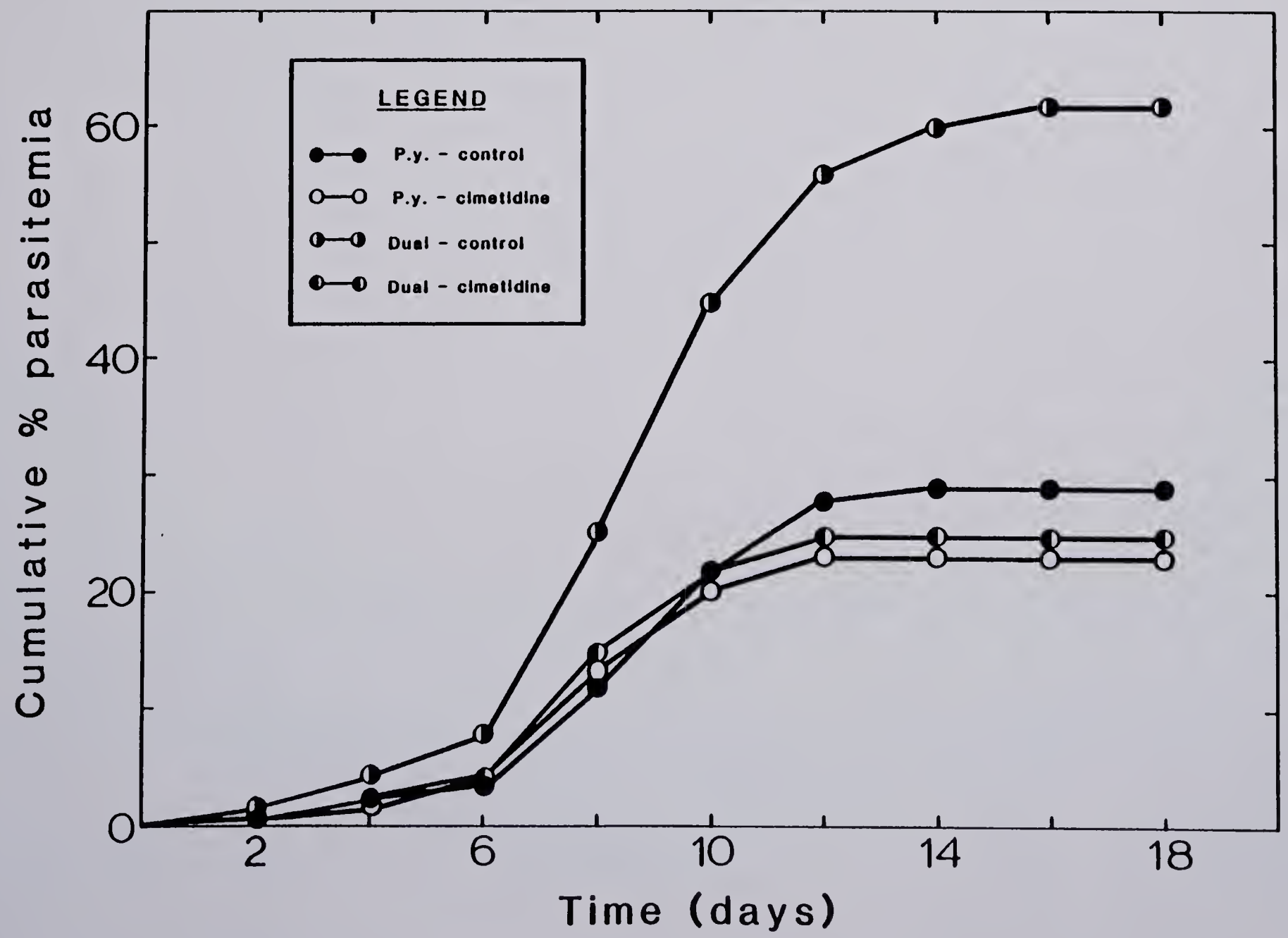

LEGEND
P.y. - control
P.y. - cimetidine
Dual - control
Dual - cimetidine
Index of malaria
30
20
10
0
2
6
10
14
18
Time (days)

Figure 31. Effect of cimetidine on the development of Plasmodium yoelii in mice infected with P. yoelii only and in mice infected with both P. yoelii and Leishmania mexicana: Cumulative percent parasitemia as a means of differentiating effects of the drug on malaria. Parasites were inoculated at Day 0.


LEGEND
P.y. - control
P.y. - cimetidine
Dual - control
Dual - cimetidine
Cumulative % parasitemia
0
20
40
60
2
6
10
14
18
Time (days)

DISCUSSION

All forms of human leishmaniasis are initially treated with either sodium stibogluconate (pentostam) or N-methyl glucanime antimonate (glucantime) (Berman 1985). However, treatment failure or relapse occurs in approximately 10-25% of all cases where these agents are used (Berman 1983). Therapeutic doses of these pentavalent antimonials are occasionally associated with the development of toxic side effects. In cases where these pentavalent antimonials are ineffective, secondary treatment consists of either pentamidine or amphotericin B. Although these agents are usually effective, their utility is offset by their relatively high toxicity, the extended treatment regimen, and the unacceptably high cost of these agents (Berman 1985). Because none of the currently used chemotherapeutic agents are completely satisfactory, intense efforts have been made to develop more effective anti-leishmanial agents. Hanson (1981) suggested that the choice of a drug for the treatment of a specific disease should be based not only on its antiparasitic activity but also on its effect on immune function.

Although we did not directly examine immune function, the results of this study indicate that the secondary activity of a given agent can affect the course of concomitant parasitic infections. Pentostam has no known immuno-modulatory activity, but is quite effective in the treatment of human leishmaniasis. However, the use of pentostam was associated with an increase in the severity of *P. yoelii* infections in BALB/c mice. On the other hand, cimetidine

modulates T suppressor cell function by inactivating the H_2 histamine receptors on these cells. Prior to this study, the antiparasitic effects of this agent had not been documented. We have previously shown that cimetidine is as effective as pentostam at limiting the progression of L. mexicana in BALB/c mice. Here we show that cimetidine is also somewhat effective at limiting P. yoelii infection in mice; moreover, the ability of this agent to reduce the severe malarial infections that develop in mice concurrently infected with L. mexicana was remarkable.

Numerous parasitic diseases are endemic in human populations throughout the tropics. Although reliable figures are not available, infection with more than one pathogen is presumably a frequent occurrence (Killick-Kendrick & Peters 1978). Parasitic diseases are usually treated with specific chemotherapeutic agents. Most of the currently used drugs exhibit functional activity against a specific parasite or against a group of closely related parasite (Rollo 1980). The effectiveness of a given agent is based on its ability to either prevent the establishment of a specific pathogen in a susceptible host or to eliminate a specific pathogen from an infected individual. Little consideration is placed on the ability of a drug to influence the course of incidental disease agents which might concomitantly or subsequently infect the host.

Agents with known parasitic activity are occasionally tested against unrelated organisms in an attempt to find new drugs for the treatment of certain diseases. Miconazole, clotrimazole, and ketoconazole were originally developed as antifungal agents; however,

these agents also exhibit *in vitro* antileishmanial activity (Berman 1981). The 8-aminoquinolines are frequently used in the treatment of malaria. Kinnamon et al. (1978) showed that an 8-aminoquinoline (WR 6026) was highly effective against visceral leishmaniasis. Chloroquine is widely used in the treatment of human malarial infections (Bygbjerg & Flachs 1986, Herzog et al. 1983, Rollo 1980). In addition to its antimalarial capabilities, chloroquine has well known anti-inflammatory properties (Rollo 1980) and can inhibit lymphocyte proliferation (Bygbjerg & Flachs 1986, Gery & Eidinger 1977, Hurvitz & Hirschhorn 1965, Panusch 1975, Salmeron & Lipsky 1983, Trist & Weatherall 1981). Treatment with chloroquine has been associated with poor antibody response to human rabies vaccine (Taylor et al. 1984), and chloroquine has a demonstrable viral inhibitory effect (Marsh et al. 1983). Chloroquine, mefloquine, and pyrimethamine are known to inhibit human natural killer cell cytotoxicity (Ferrante & Goh 1984). Somewhat surprisingly, similar studies with other chemotherapeutic agents have rarely been conducted.

It is evident from this study that the choice of a chemotherapeutic agent can have important effects on both the primary pathogen and on secondary parasites. Given the presumed incidence of multiple infections in human populations in the tropics, greater emphasis should be placed on determination of the parasite burden present in each patient. More appropriate therapeutic measures can then be undertaken.

Chapter VII

INTERACTIONS BETWEEN MALARIA (*PLASMODIUM YOELII*) AND LEISHMANIASIS (*LEISHMANIA MEXICANA AMAZONENSIS*): AFFECT OF CONCOMITANT INFECTION ON PARAMETERS AFFECTING DISEASE TRANSMISSION.

Introduction

Arthropod transmitted diseases depend on vector blood-feeding behavior for transmission. Initial contact with an infected host, completion of an extrinsic pathogen incubation period, and subsequent feeding on a susceptible vertebrate are implicit to this process. Factors which modulate any aspect of blood-feeding behavior can affect pathogen transmission.

Mosquito engorgement patterns are often assumed to reflect innate cycles of "hunger" and preference for particular hosts (Washino & Tempelis 1967, Tempelis et al. 1970). However, evidence suggests that feeding patterns may also be modulated by differences in host defensive behavior (Edman & Kale 1971). Many birds and rodents utilize various defensive regimes to prevent mosquitoes from successfully engorging (Day & Edman 1984b, Edman & Kale 1971, Edman et al. 1974, Walker & Edman 1985 1986). In spite of this, a number of mosquito-borne diseases are enzootic in these hosts, suggesting that

some, as yet unknown, factor(s) allow mosquitoes to feed on these animals.

Laboratory mice use various defensive movements to prevent hematophagous insects from obtaining blood. Day and Edman (1983 1984b) and Day et al. (1984) found that mice infected with Plasmodium berghei, P. chabaudi, or P. yoelii were less effective at preventing mosquitoes from obtaining blood than their uninfected counterparts. Blood uptake occurred most frequently when gametocytes were prevelant. Modification of host behavior by these parasites presumably facilitates pathogen transmission.

We have previously shown that mice infected with both Leishmania mexicana and P. yoelii develop more severe infections than mice infected with either parasite alone (Chapters 2 and 3). The purpose of this study was to determine if concurrent infection with these pathogens would affect host behavior. Core body temperature, daily activity patterns and mosquito engorgement success were the parameters used to assay for behavioral modification. Although Aedes aegypti is not a natural vector of either P. yoelii or L. mexicana, it served as a bioassay with which to quantify behavioral changes in mice infected with these pathogens.

Materials and Methods

Animals

Female BALB/c mice were obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts. Mice weighed 25-30 grams and were 14 weeks old at the start of experiments.

Malaria

The 17x strain of *Plasmodium yoelii* was used. Procedures for the maintanence and inoculation of *P. yoelii* have been described (Chapter 2). Mice were infected with 1×10^6 parasites.

Leishmaniasis

Leishmania mexicana amazonensis (Walter Reed strain 227) was used. The source and history of this isolate have been described (Nolan et al. 1984), as have procedures for the maintanence and inoculation of the parasite (Chapter 2). Mice were infected with 1×10^6 amastigotes.

Experimental design

Four groups of 15 mice each were used. One group was sham injected with 0.5 ml PBS and served as a control. A second group was inoculated with *P. yoelii*, a third with *L. mexicana*, and the fourth with both *P. yoelii* and *L. mexicana*.

Determination of parasitemia

The course of the malaria infection was monitored by determining *P. yoelii* parasitemia in Giemsa-stained blood smears (Chapter 2).

Blood smears were prepared every two days until no parasites were found over a six day period. Daily percent parasitemia was plotted for each group of mice infected with P. yoelii.

Leishmania mexicana infections were monitored weekly by recording the thickness of the infected foot and the contralateral uninfected foot using a direct-reading vernier caliper. Measurements commenced 1 week prior to the inoculation of L. mexicana and continued throughout the course of the experiment. The size of the lesion was calculated by subtracting the measurement (in mm) obtained from the uninfected footpad from that of the infected footpad. Mean values were recorded for each group of mice.

Mosquitoes

Aedes aegypti aegypti (L.) (Rockefeller Strain) were maintained in the laboratory at 27°C, 70-80% RH, and a 16L:8D regime. Cohorts of 1,500 to 2,000 day-old mosquitoes were maintained in screened cages. Seven-day old mosquitoes from a given cohort were cold anesthetized and sorted by sex. Groups of 10 female mosquitoes were placed in screen-topped, one-pint ice-cream cartons. These cartons served as holding cages prior to the commencement of experiments. All mosquitoes were provided with 3% sucrose. Experiments were conducted using 8 to 10 day old A. aegypti.

Mosquito feeding experiments

Aedes aegypti from the pint holding cartons were aspirated into one gallon ice-cream containers with screened lids using a mechanical aspirator. Only water was provided while mosquitoes were in these

containers. Experiments commenced 24 hr after the introduction of mosquitoes into the gallon containers. All experiments were conducted late in the afternoon under fluorescent lights.

Experiments lasted 45 min and were initiated with the introduction of a single mouse into the cage. Unrestrained (UR) mice and restrained mice from each of the 4 experimental groups were used. A total of 7 trials each were conducted with UR mice which were uninfected or which were infected with L. mexicana. Ten trials were conducted with UR mice which were infected with P. yoelii and 10 with mice infected with both P. yoelii and L. mexicana. Restrained mice were anesthetized with nembutal (0.25 ml of a solution made up of 3 parts nembutal to 7 parts physiological saline) 10 min prior to their introduction into the cage. These mice served as controls for each of the 4 experimental groups. Preliminary data indicated that mosquitoes fed equally well on restrained mice from each of the 4 experimental groups. In light of this, only 2 control trials were conducted using restrained mice from each of the 4 experimental groups and data from these 8 tests were pooled.

Mice were removed from the cages after 45 min and the mosquitoes were aspirated back into the holding cartons using a mechanical aspirator. The surviving mosquitoes were killed by placing these containers into a freezer, and the mosquitoes were then counted and checked for the presence or absence of blood.

Activity cage experiments

Mice from each of the four experimental groups were individually placed in activity cages (Wahmann Manufacturing Co.). Each activity cage consisted of a wheel fitted with an automatic counter which recorded each half revolution in either direction. The daily number of revolutions were recorded for each activity wheel during a 7 day control period and for a 3 week period following the inoculation of parasites. The experiment was replicated 4 times. The average number of revolutions run by each group of mice was calculated and plotted.

Core body temperature

A YSI electronic tele-thermometer fitted with an electronic probe was used to monitor the core body temperatures of 10 mice from each experimental group. Temperatures were recorded every other day over the course of the experiment. Mean core body temperatures for each group of mice were calculated and plotted.

Results

Determination of parasitemia

Mice which were inoculated with P. yoelii developed infections in which approximately 15% of the RBC were infected during peak parasitemia (Fig. 32). Plasmodium yoelii parasitemia rates were significantly enhanced when mice were concurrently infected with both P. yoelii and L. mexicana. Approximately 35% of the RBC were infected in these mice during the height of the infection (Fig. 32).

These results correspond with those previously obtained during experiments on dual infections in BALB/c mice (Chapters 2 and 3).

Mice infected with L. mexicana and P. yoelii did not develop lesions which were significantly larger or smaller than those which occurred in mice only infected with L. mexicana (because of this, data for the progression of L. mexicana infection are not presented). However, previous evidence (chapter 2) indicated that lesions in BALB/c mice only become detectable 3 weeks after parasite inoculation. We have previously demonstrated that mice infected with both L. mexicana and P. yoelii develop lesions which are significantly larger than those lesions which occur in mice only infected with L. mexicana (Chapters 2 and 3). Such differences normally become detectable within 5 weeks of parasite inoculation. Therefore, it was not surprising that differences in lesion development between these two groups of mice were not detected.

Mosquito feeding experiments

The mean number of A. aegypti remaining in each cage each day is shown in Figs. 33-35. More mosquitoes were recovered alive from cages containing restrained mice than from cages containing unrestrained animals. Although direct observations were not made, it is assumed that differences in mosquito mortality rates between control and experimental cages resulted primarily from defensive movements of unrestrained mice. Day (1981) reported that laboratory mice caught and ate mosquitoes. Some mortality which occurred in cages which contained unrestrained mice might have resulted from factors other

than host defensive behavior. Close examination of the cages occasionally revealed the presence of dead mosquitoes which were "trapped" in excrement voided by unrestrained mice. None of the restrained mice produced urine or feces while anesthetized. Although we examined each cage for dead mosquitoes when the mice were removed from the cages, dead mosquitoes may have been consumed by the animals prior to our examination. It is therefore impossible to determine the exact cause of observed mortality differences.

Mosquito engorgement success on mice from each experimental group is shown in Figs. 36-38. A. aegypti were unable to obtain blood from uninfected mice (Fig. 36) or from mice infected only with L. mexicana (Fig. 37). A small number of mosquitoes obtained blood from mice infected with P. yoelii only. Significant engorgement rates only occurred during peak parasitemia; however, less than 20% of the mosquitoes obtained blood during this period. Mosquitoes also obtained blood from mice infected with both P. yoelii and L. mexicana (Fig. 38). Almost 50% of the mosquitoes which were exposed to dually infected mice obtained blood during the period of peak parasitemia. Again, mosquito engorgement only occurred during this period.

Activity cage experiments

The activity patterns of mice from each of the 4 experimental groups are shown in Figs. 39-41. The number of revolutions ran by each mouse increased during the initial acclimation period (Figs 39-41). The animals presumably became accustomed to the activity wheels during this time. Uninfected mice normally ran at least 5,000

revolutions per wheel per day (Figs. 39-41). A great deal of variation in daily activity occurred over the course of the experiment. Mice infected with P. yoelii became less active as the infection progressed. These animals ran an average of 1,500 revolutions per day during the peak of the infection. Daily activity gradually increased following clearance of the parasites from the peripheral circulation (Fig. 39). Mice infected with L. mexicana remained as active as the uninfected mice throughout the experiment (Fig. 40). The daily activity patterns of mice infected with both P. yoelii and L. mexicana resembled those of mice only infected with P. yoelii; however, running on the activity wheel decreased almost entirely during peak Plasmodium infection (Fig. 41). This reduction in running was both more pronounced and of a longer duration than changes which occurred to mice infected with P. yoelii only.

Core body temperature

Uninfected mice maintained a core body temperature of approximately 36.5 - 37.0°C throughout the course of the experiment (Figs. 42-44). Mice infected with L. mexicana initially maintained this core body temperature. However, these mice gradually became hyperthermic as the infection progressed. The core body temperature of these mice had stabilized between 37.5°C and 38.0°C at the conclusion of the experiment (Fig. 43). Mice infected with P. yoelii became slightly hypothermic at the peak of the infection (Fig. 42) as did mice infected with both P. yoelii and L. mexicana (Fig. 44).

Hypothermia in dually infected mice was more pronounced than that observed in mice infected only with P. yoelii (Figs. 42 & 44).

Figure 32. Plasmodium yoelii parasitemia in BALB/c mice infected with a) P. yoelii only (PYIM), and b) both P. yoelii and Leishmania mexicana (DIM). Parasites were inoculated on Day 0 (+/- SE).

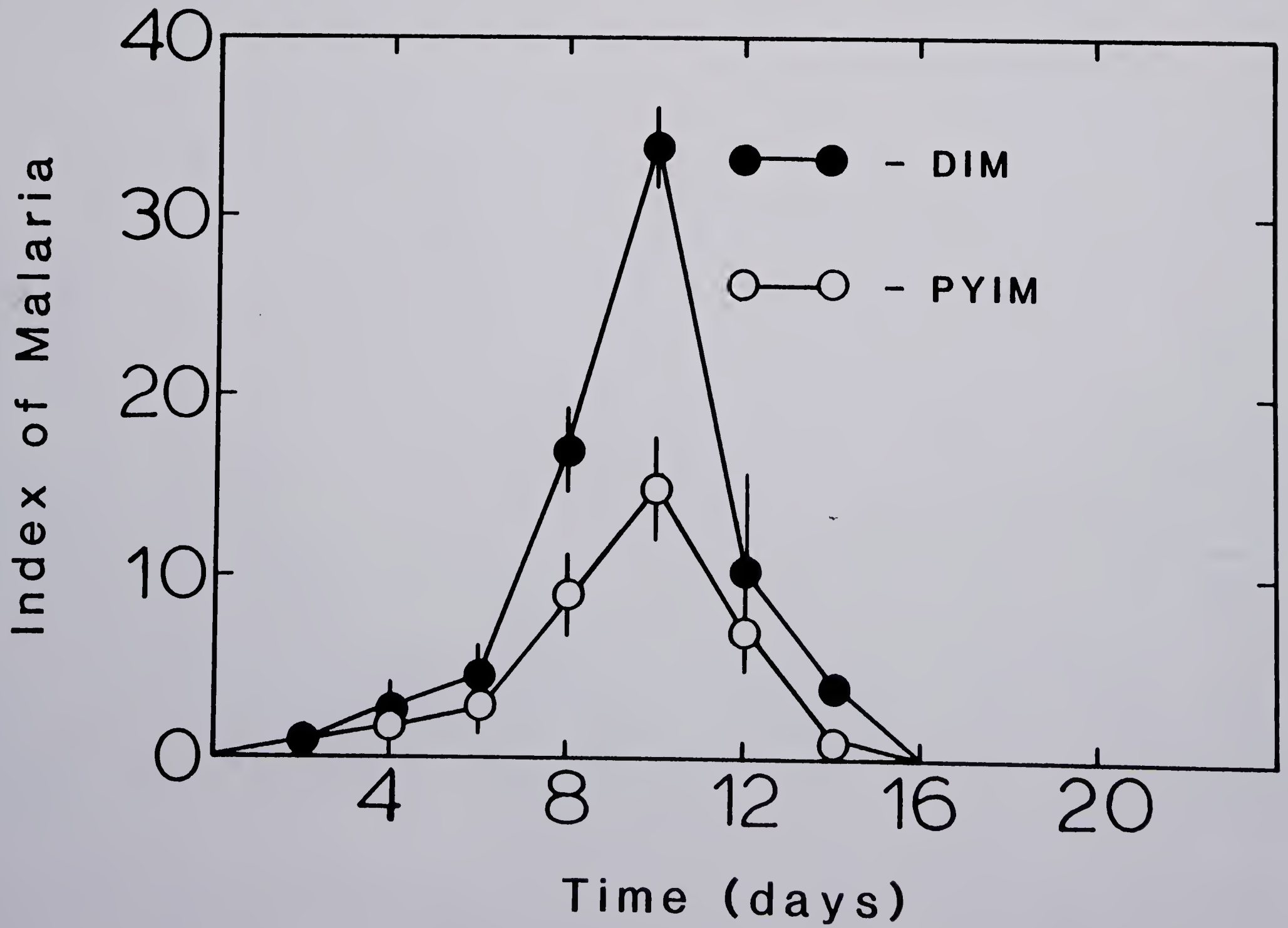

40
30
20
10
0
4
8
12
16
20
- DIM
- PYIM
Index of Malaria
Time (days)

Figure 33. Mean number of Aedes aegypti remaining after exposure to a) restrained mice (RM), b) unrestrained uninfected mice (UR-UIM), and c) unrestrained Plasmodium yoelii infected mice (UR-PYIM). Parasites were inoculated on Day 0 (+/- SE).

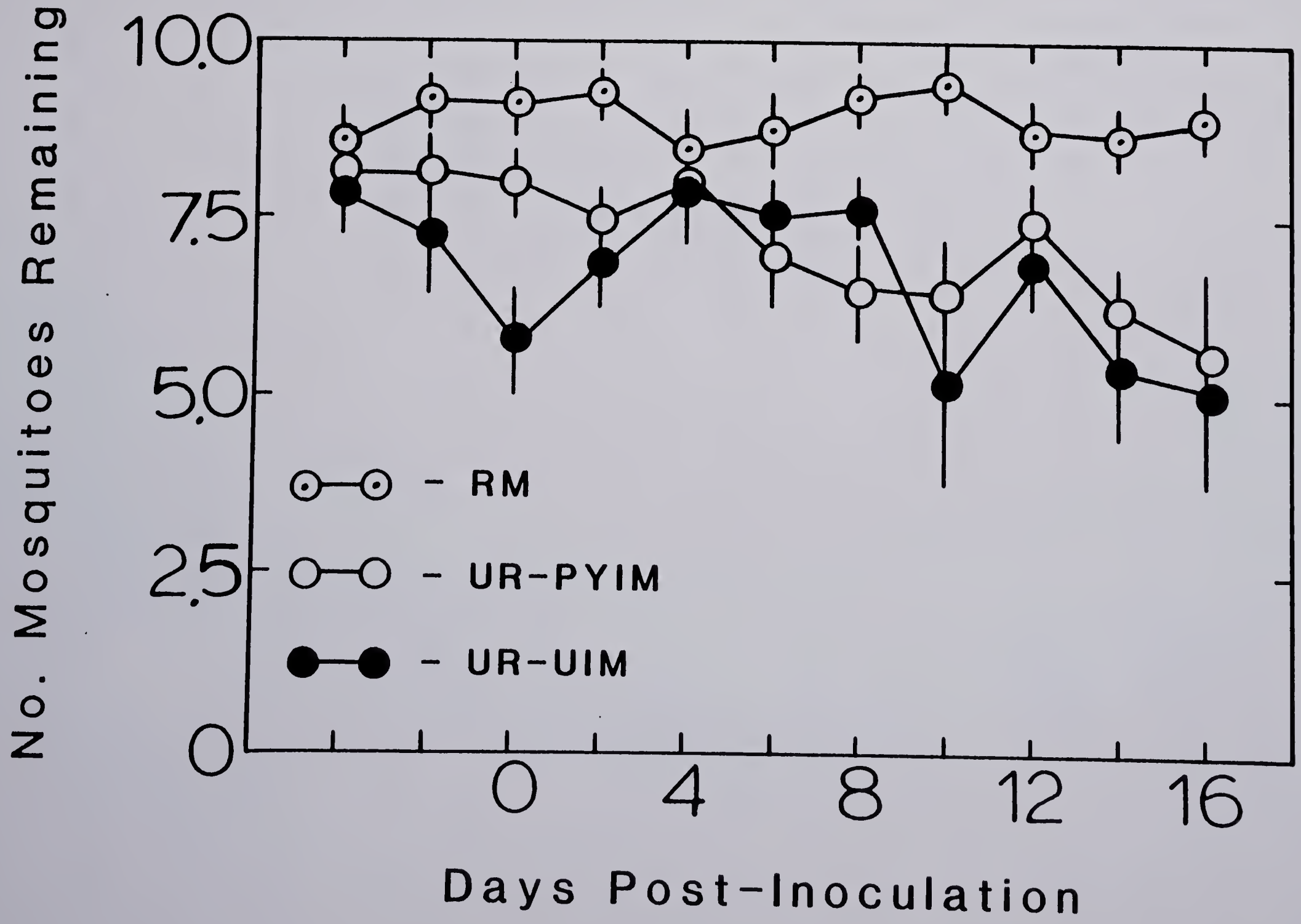

No. Mosquitoes Remaining
10.0
7.5
5.0
2.5
0
RM
UR-PYIM
UR-UIM
0
4
8
12
16
Days Post-Inoculation

Figure 34. Mean number of Aedes aegypti remaining after exposure to a) restrained mice (RM), b) unrestrained uninfected mice (UR-UIM), and c) unrestrained Leishmania mexicana infected mice (UR-LMIM). Parasites were inoculated on Day 0 (+/- SE).

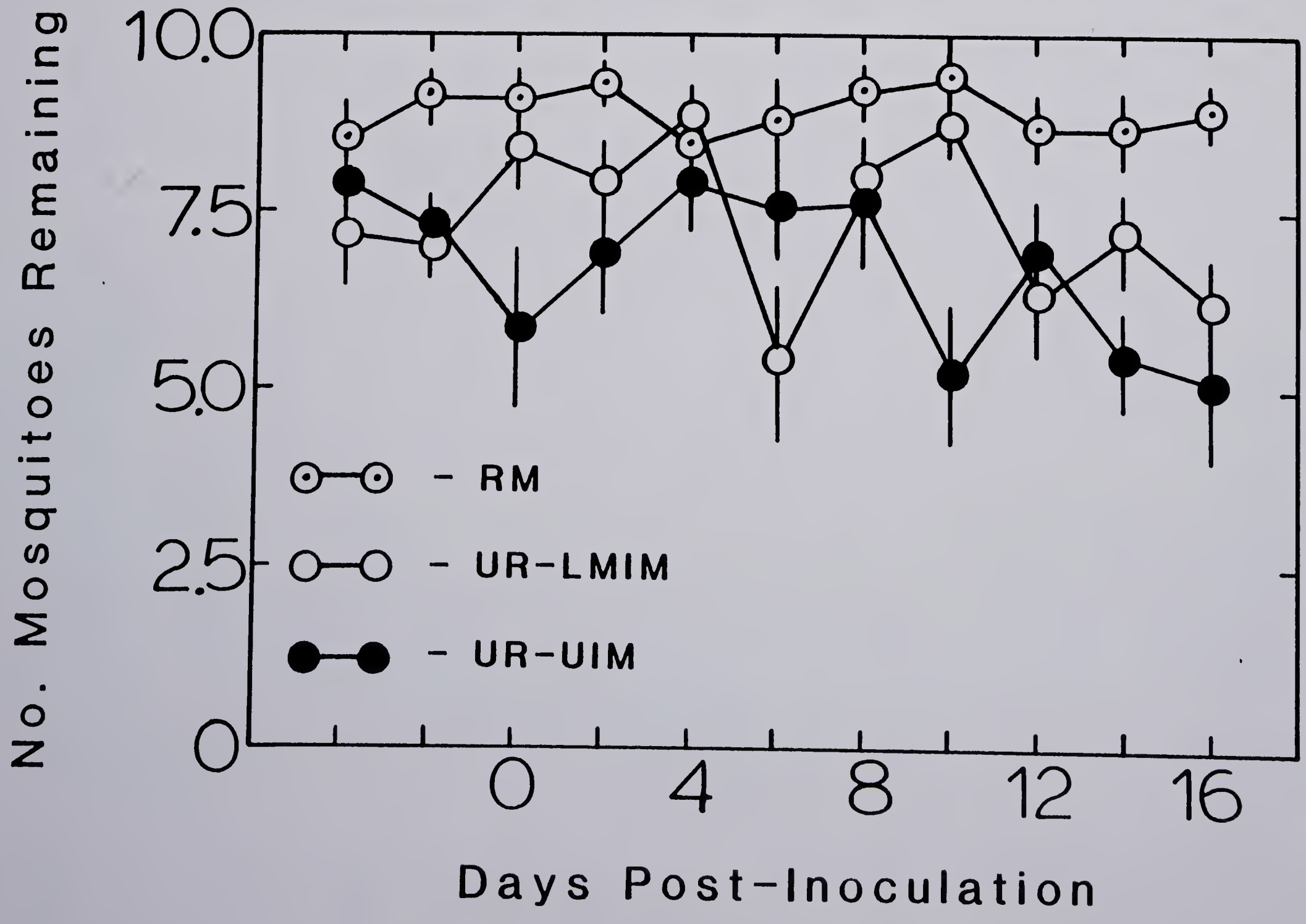

No. Mosquitoes Remaining
10.0
7.5
5.0
2.5
0
RM
UR-LMIM
UR-UIM
0
4
8
12
16
Days Post-Inoculation

Figure 35. Mean number of Aedes aegypti remaining after exposure to a) restrained mice (RM), b) unrestrained uninfected mice (UR-UIM), and c) unrestrained mice infected with both Plasmodium yoelii and Leishmania mexicana (UR-DIM). Parasites were inoculated on Day 0 (+/- SE).

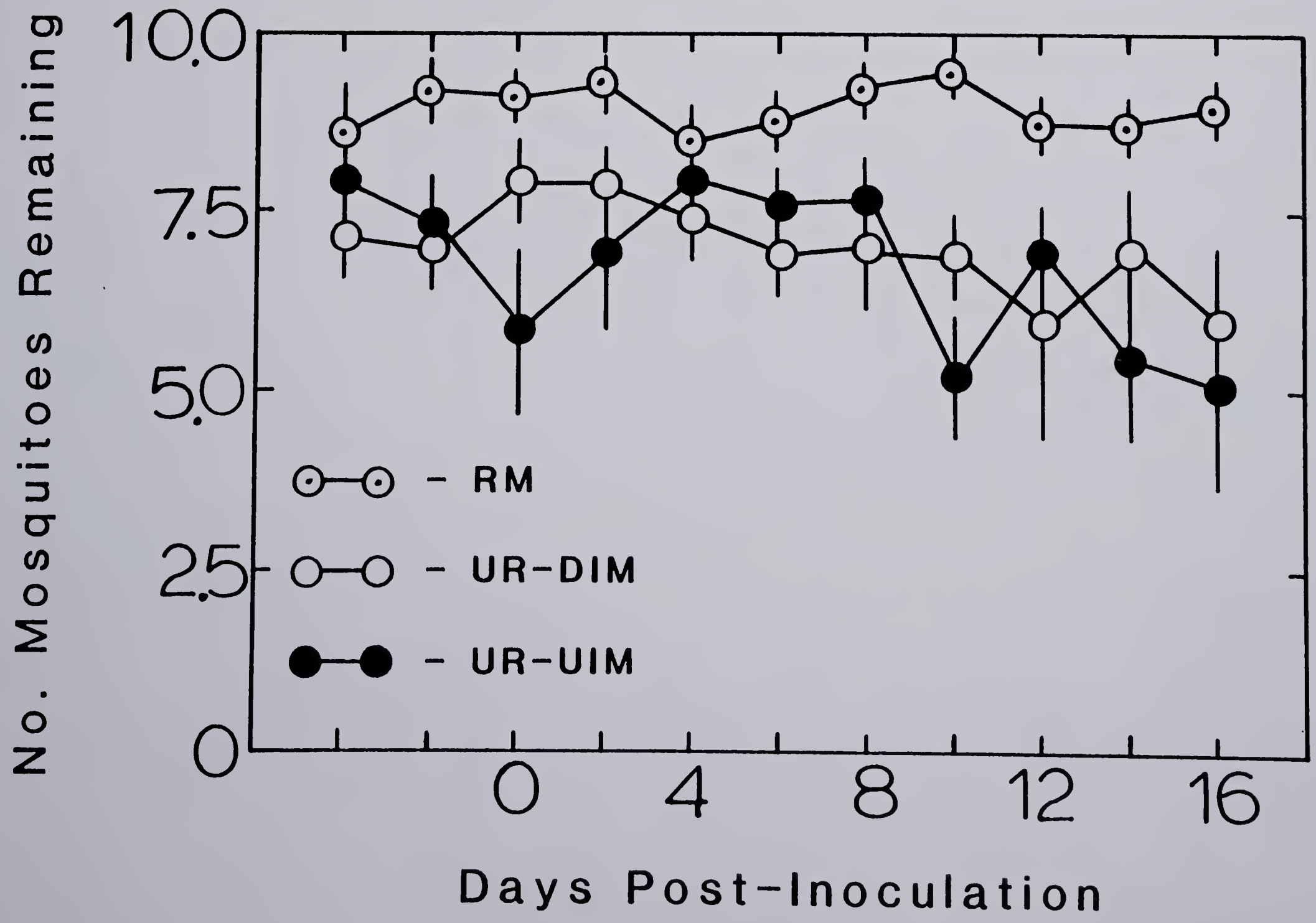

No. Mosquitoes Remaining
10.0
7.5
5.0
2.5
0
RM
UR-DIM
UR-UIM
0
4
8
12
16
Days Post-Inoculation

Figure 36. Feeding success of Aedes aegypti when exposed to a) restrained mice (RM), b) unrestrained, uninfected mice (UR-UIM), and c) unrestrained Plasmodium yoelii infected mice (UR-PYIM). Parasites were inoculated on Day 0 (+/- SE).

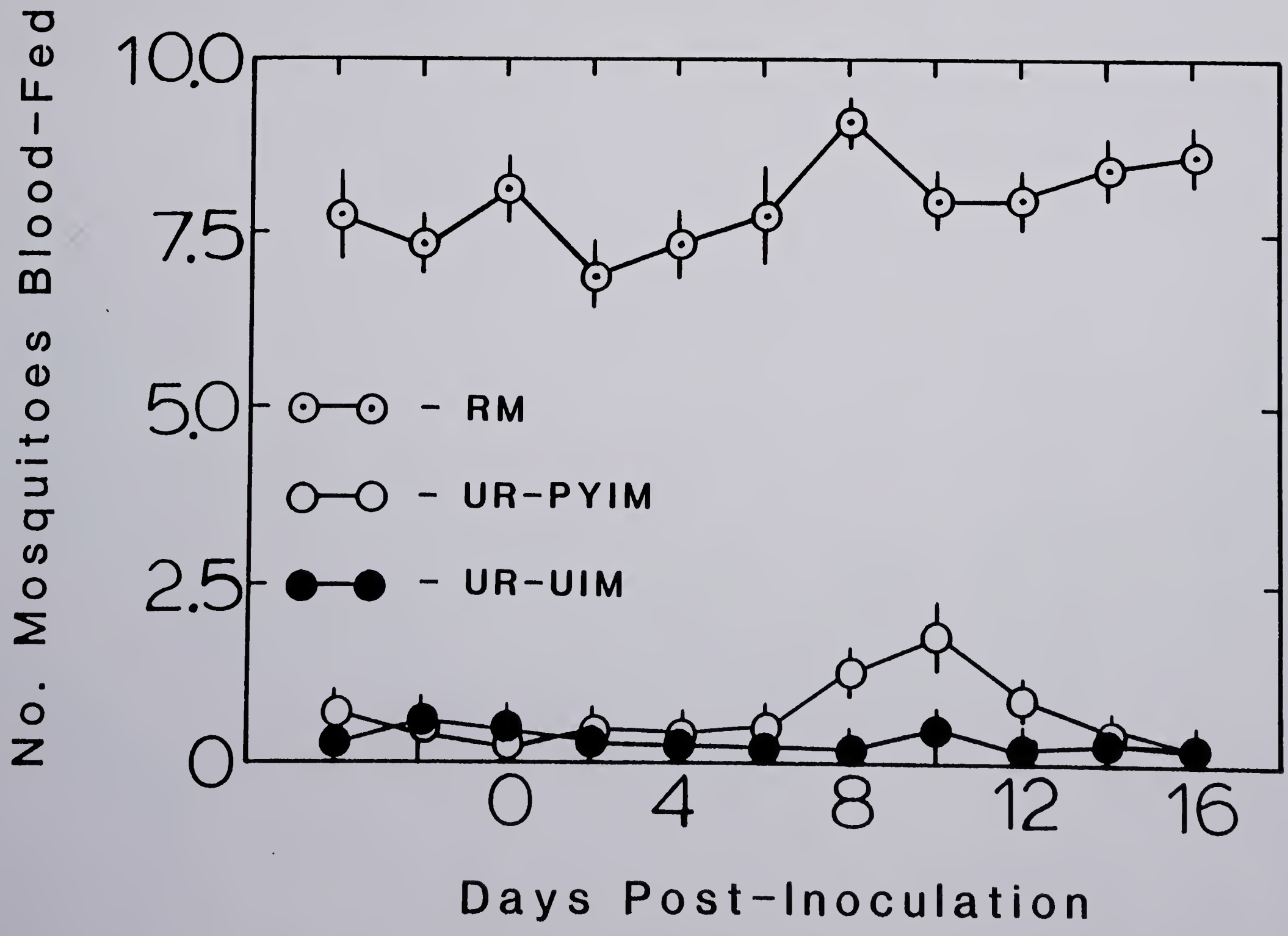

No. Mosquitoes Blood-Fed
10.0
7.5
5.0
2.5
0
RM
UR-PYIM
UR-UIM
0
4
8
12
16
Days Post-Inoculation

Figure 37. Feeding success of Aedes aegypti when exposed to a) restrained mice (RM), b) unrestrained, uninfected mice (UR-UIM), and c) unrestrained Leishmania mexicana infected mice (UR-LMIM). Parasites were inoculated on Day 0 (+/- SE).

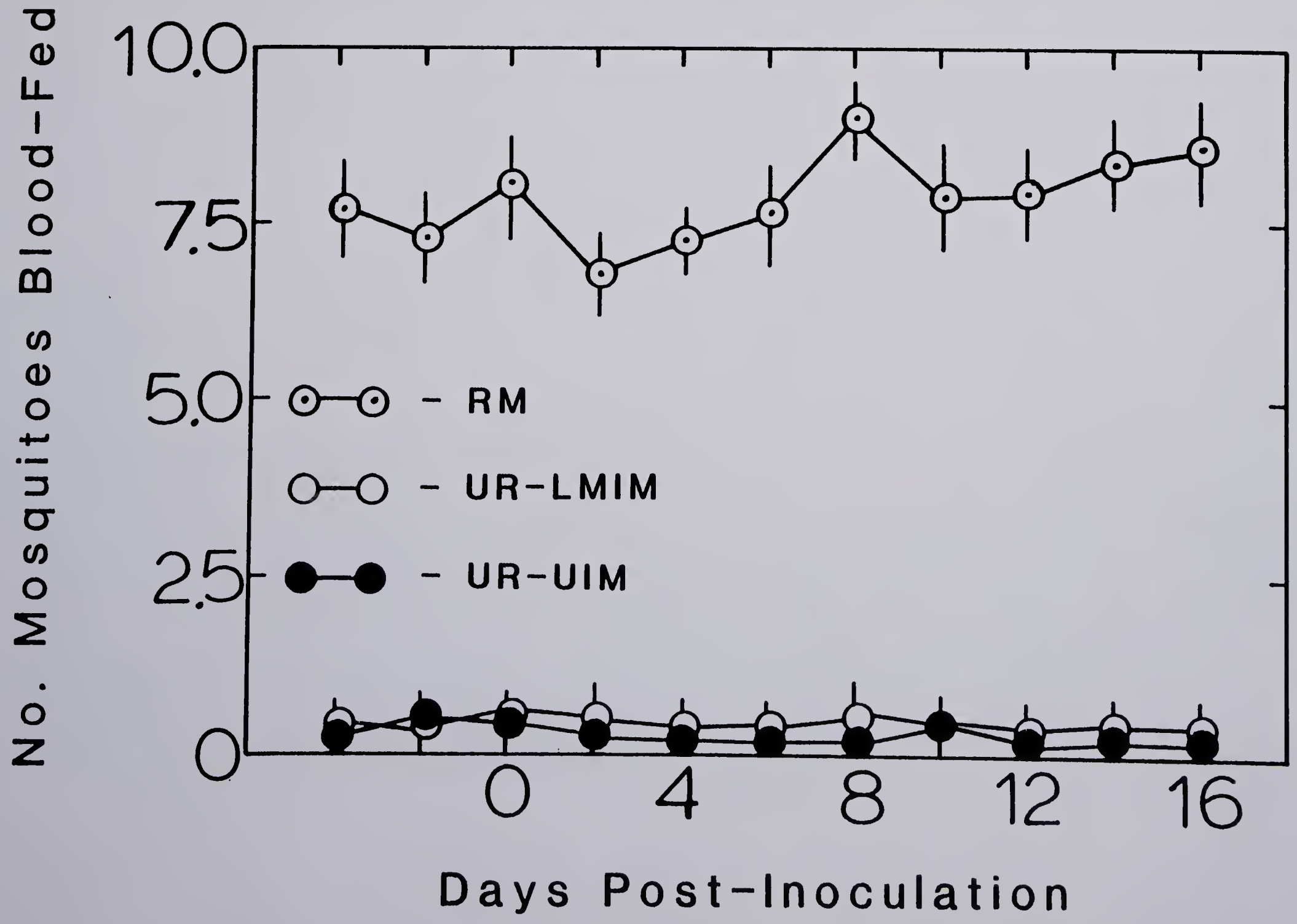

No. Mosquitoes Blood-Fed
10.0
7.5
5.0
2.5
0
- RM
- UR-LMIM
- UR-UIM
0
4
8
12
16
Days Post-Inoculation

Figure 38. Feeding success of Aedes aegypti when exposed to a) restrained mice (RM), b) unrestrained, uninfected mice (UR-UIM), and c) unrestrained mice infected with both Plasmodium yoelii and Leishmania mexicana (UR-DIM). Parasites were inoculated on Day 0 (+/- SE).

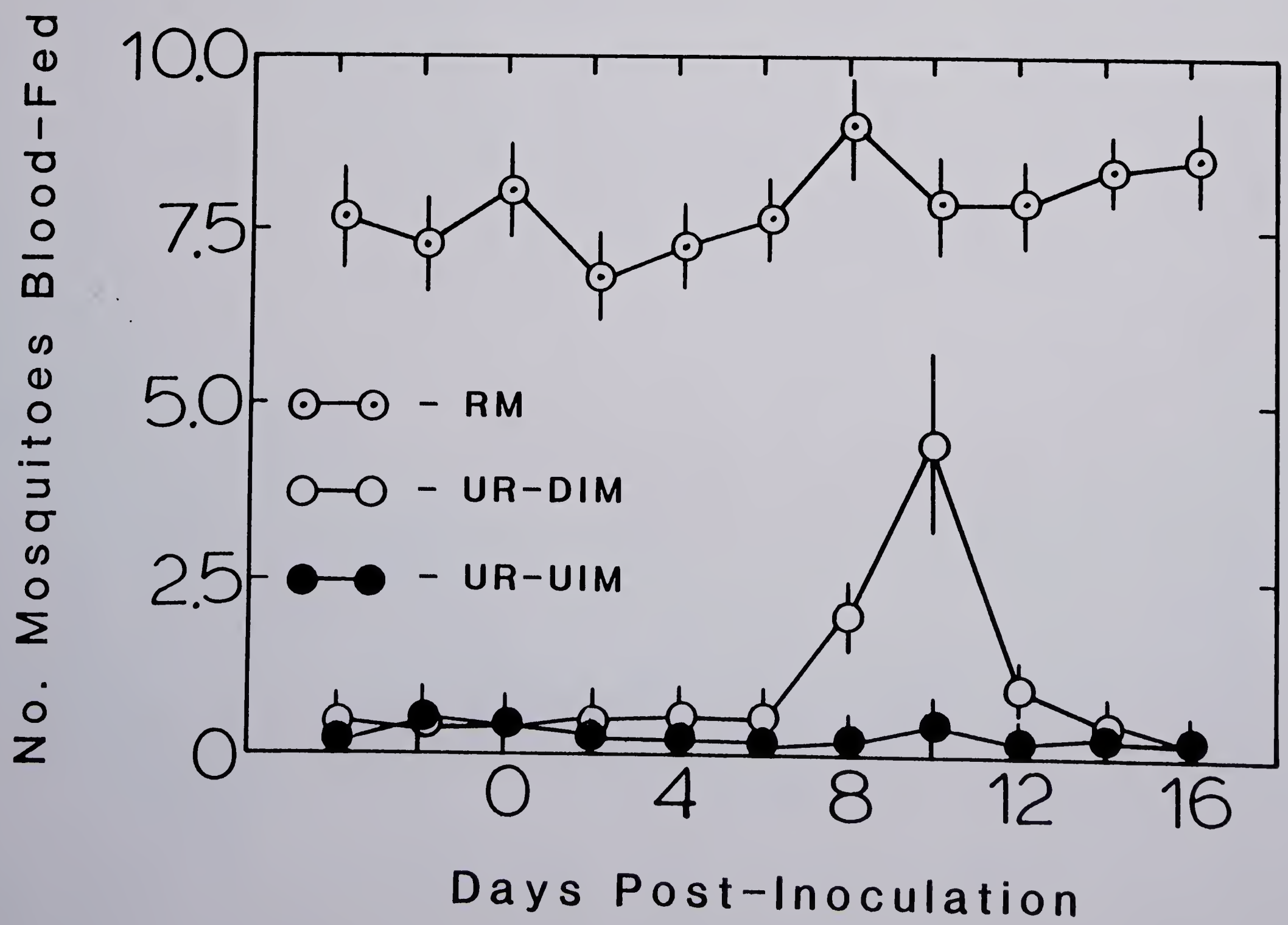

No. Mosquitoes Blood-Fed
10.0
7.5
5.0
2.5
0
- RM
- UR-DIM
- UR-UIM
0
4
8
12
16
Days Post-Inoculation

Figure 39. Mean daily activity of a) uninfected mice (UIM), and b) Plasmodium yoelii infected mice (PYIM). Parasites were inoculated on Day 0 (+/- SE).

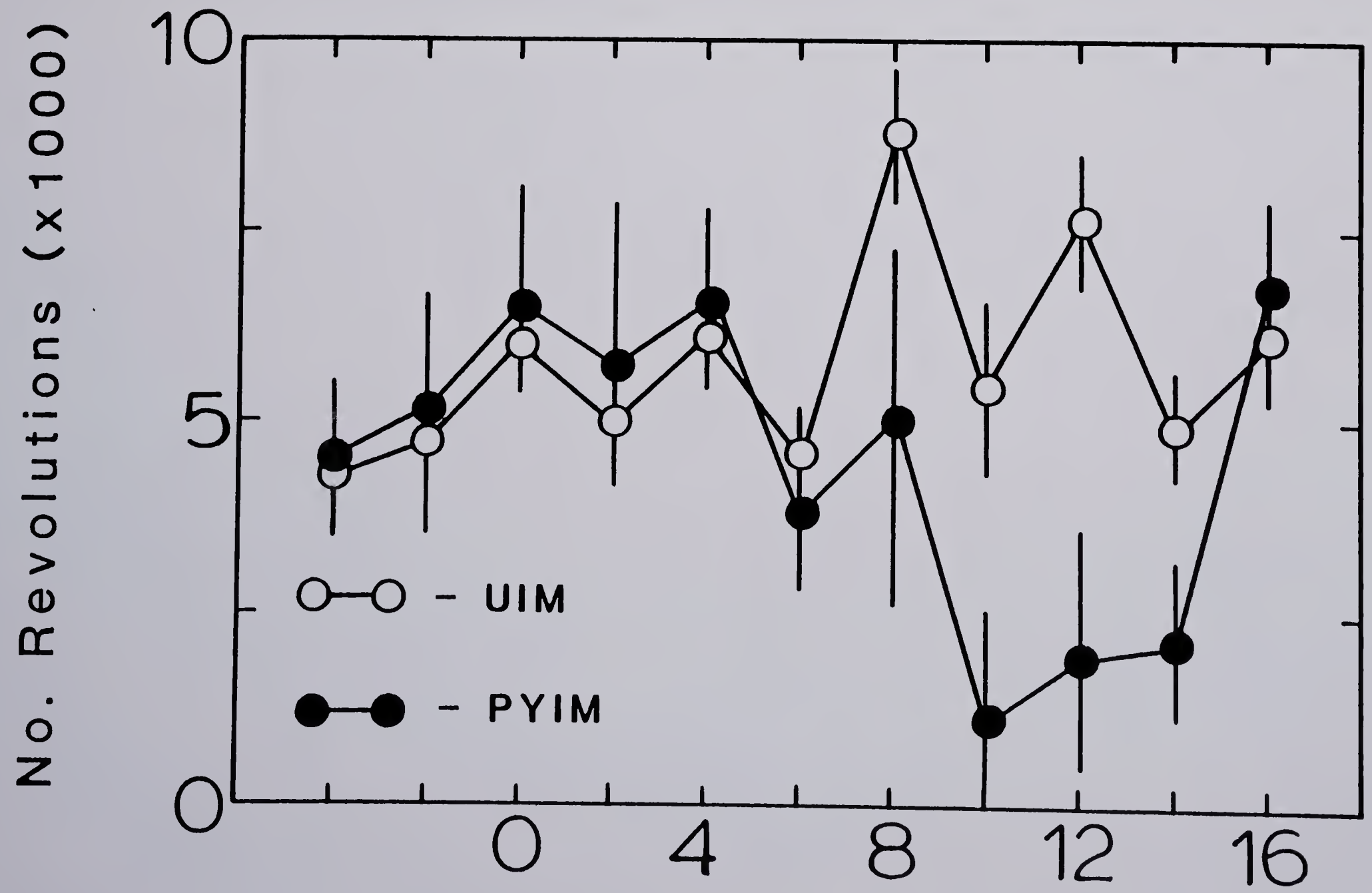

No. Revolutions (x1000)
10
5
0
UIM
PYIM
0
4
8
12
16
Days Post-Inoculation

Figure 40. Mean daily activity of a) uninfected mice (UIM), and b) Leishmania mexicana infected mice (LMIM). Parasites were inoculated on Day 0 (+/- SE).

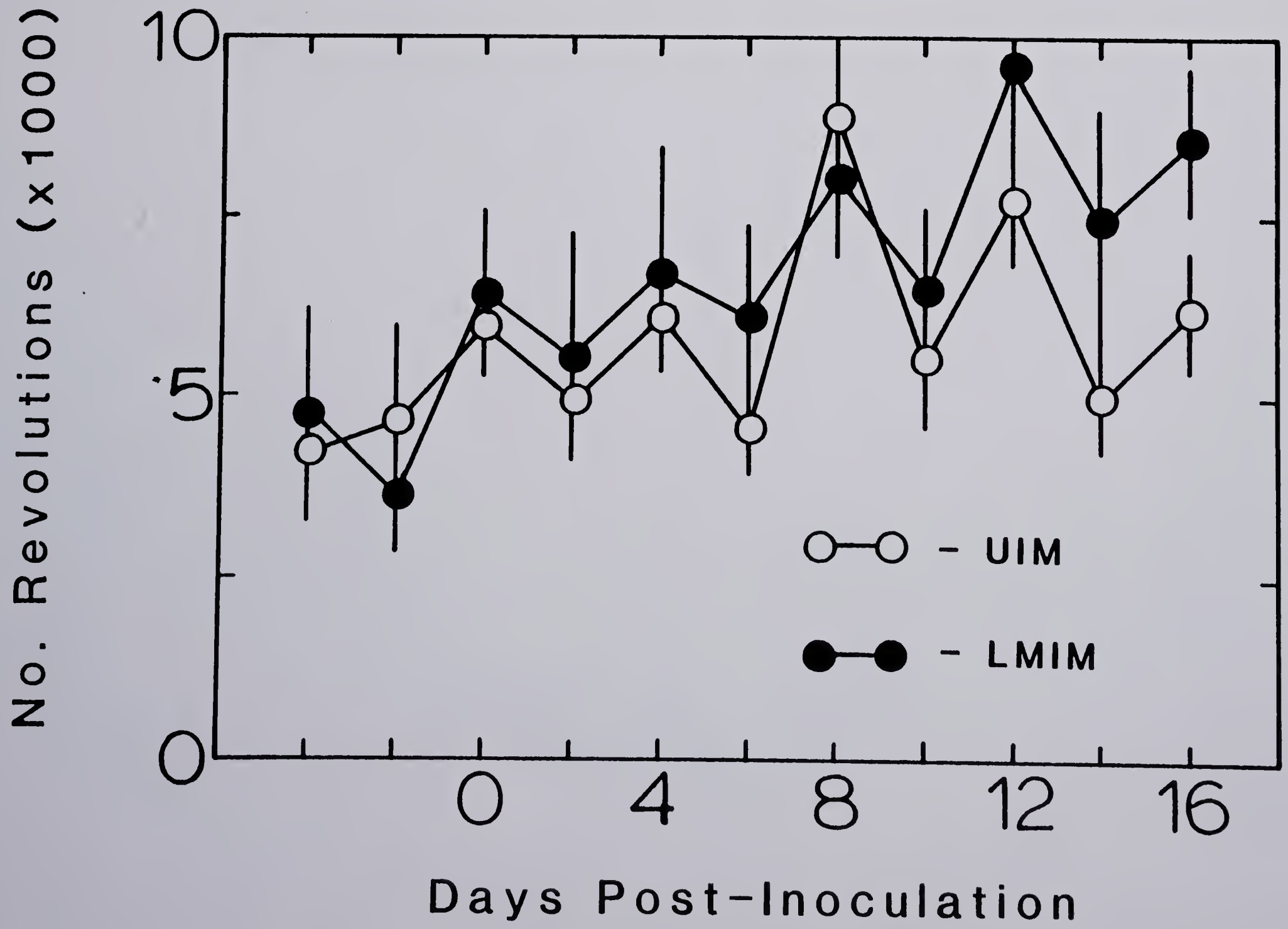

No. Revolutions (x1000)
10
5
0
0
4
8
12
16
Days Post-Inoculation
- UIM
- LMIM

Figure 41. Mean daily activity of a) uninfected mice (UIM), and b) mice infected with both Plasmodium yoelii and Leishmania mexicana (DIM). Parasites were inoculated on Day 0 (+/- SE).

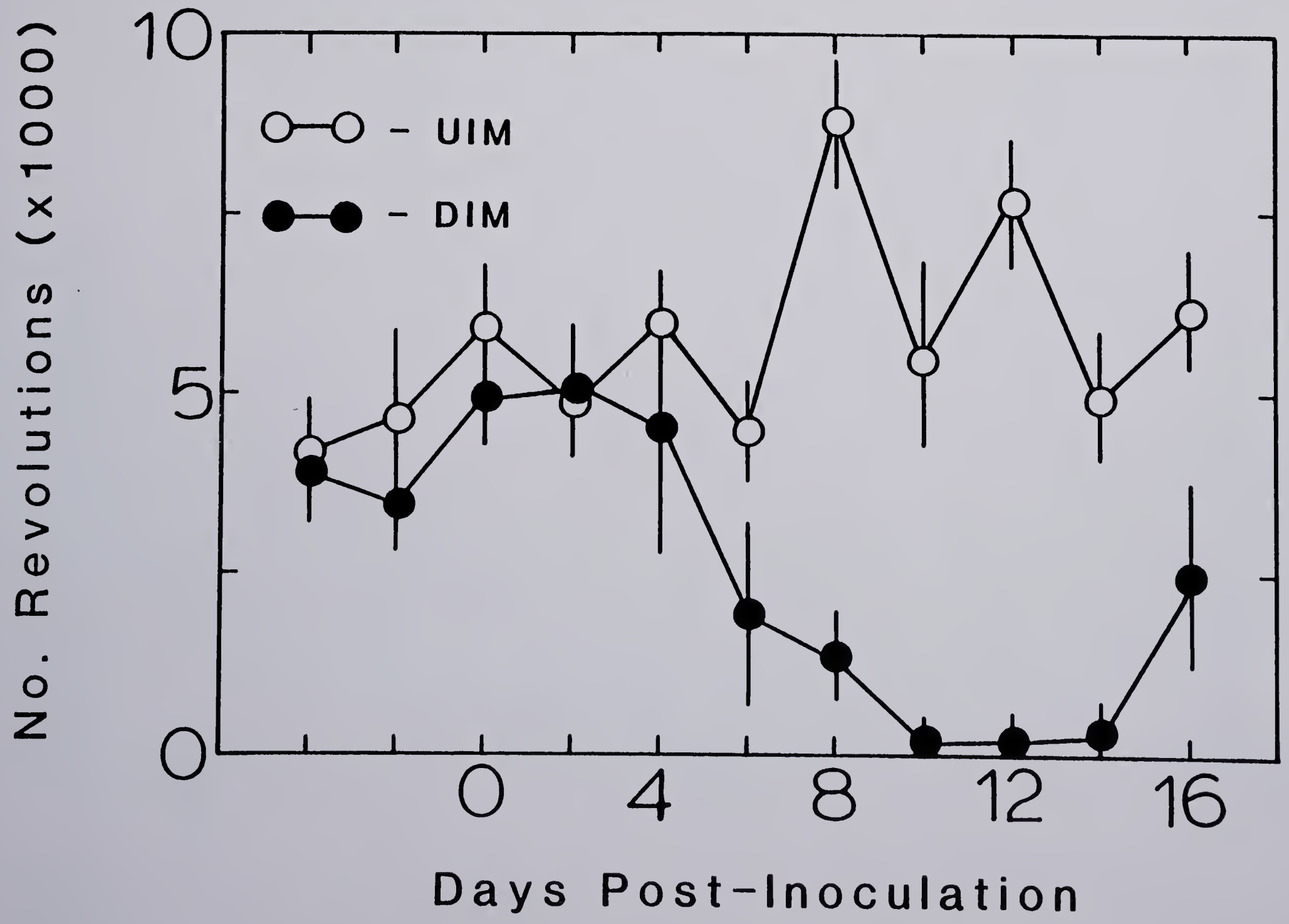

10
5
0
No. Revolutions (x1000)
UIM
DIM
0
4
8
12
16
Days Post-Inoculation

Figure 42. Mean body temperature of a) uninfected mice (UIM), and b) Plasmodium yoelii infected mice (PYIM). Parasites were inoculated on Day 0 (+/- SE).

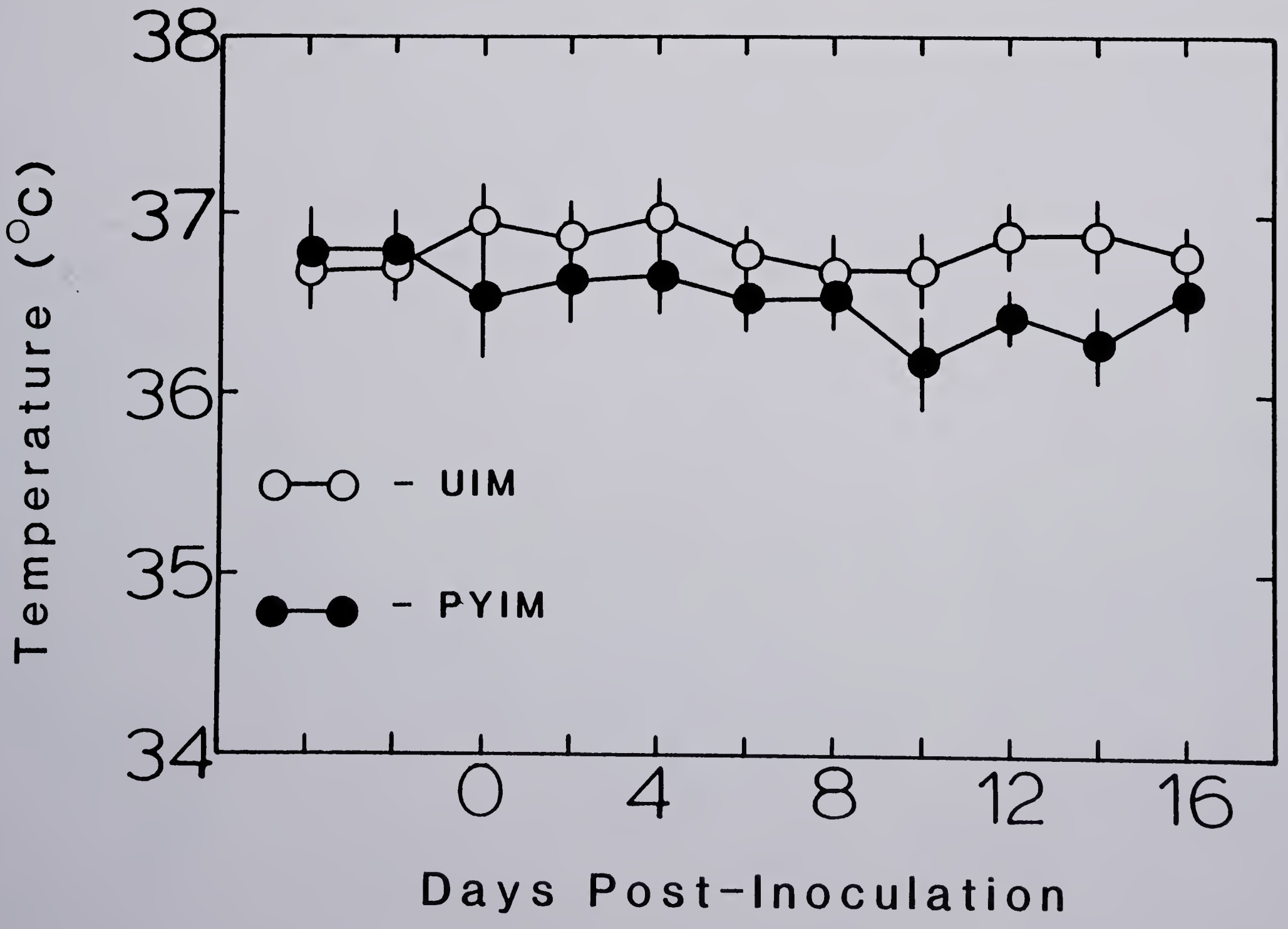

38
37
36
35
34
Temperature (°C)
UIM
PYIM
0
4
8
12
16
Days Post-Inoculation

Figure 43. Mean body temperature of a) uninfected mice (UIM), and b) Leishmania mexicana infected mice (LMIM). Parasites were inoculated on Day 0 (+/- SE).

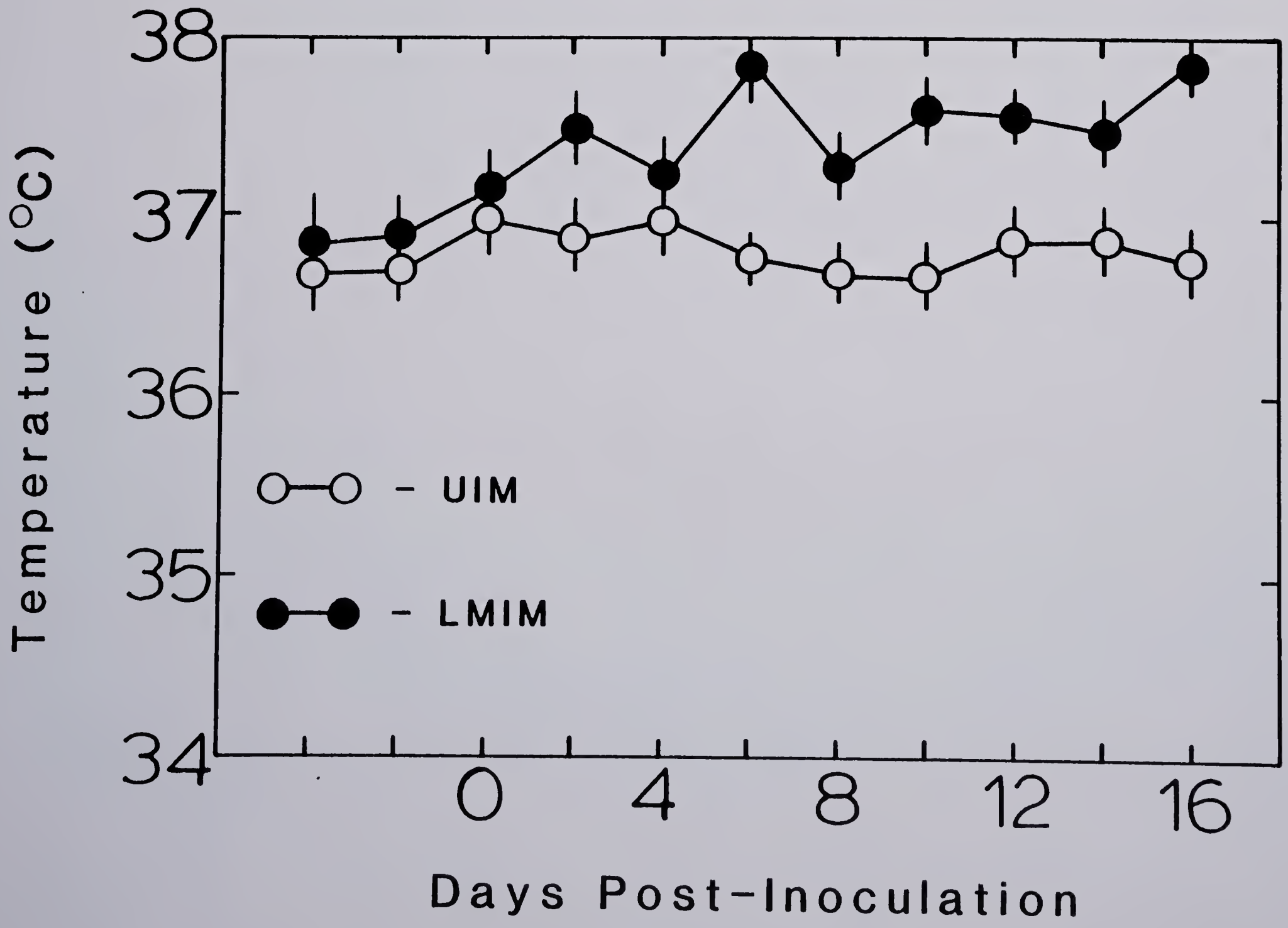
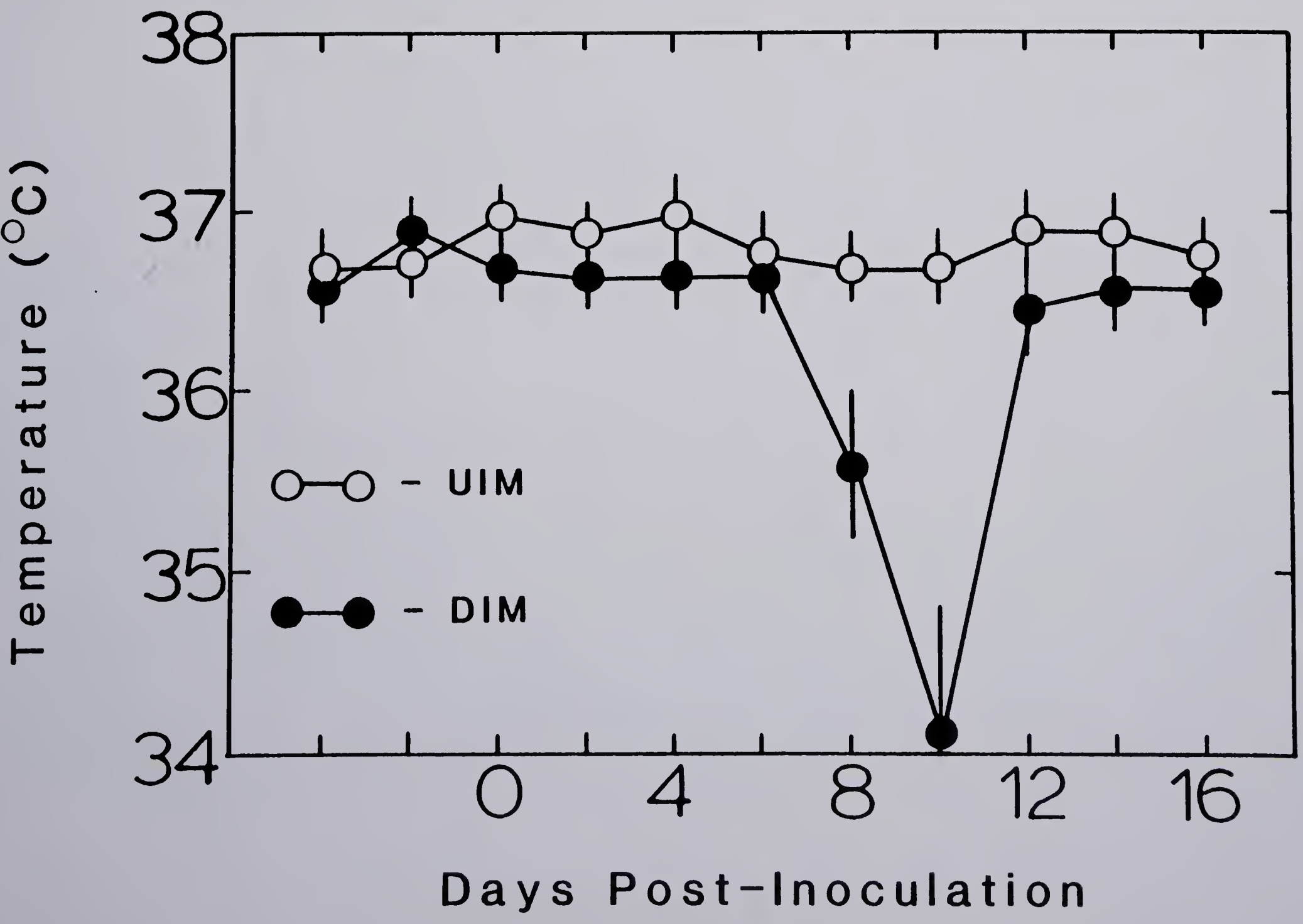

38
37
36
35
34
0
4
8
12
16
Temperature (°C)
Days Post-Inoculation
- UIM
- LMIM

Figure 44. Mean body temperature of a) uninfected mice (UIM), and b) mice infected with both Plasmodium yoelii and Leishmania mexicana (DIM). Parasites were inoculated on Day 0 (+/- SE).


38
37
36
35
34
Temperature (°C)
UIM
DIM
0
4
8
12
16
Days Post-Inoculation

Discussion

Numerous studies have shown that parasite manipulation of host/vector interplay can result in facilitated pathogen transmission (Molyneux & Jefferies 1986). Parasites can affect the probing ability of infected insects (Anez & East 1984, Beach et al. 1985, Jenni et al. 1980, Rossignol et al. 1984), and certain arthropods locate blood from infected hosts more rapidly than from uninfected animals (Rossignol et al. 1985). Pathogen-mediated alteration of host behavior can increase vector engorgement rates on parasitemic hosts (Day & Edman 1984b, Day et al. 1983).

Day and Edman (1983) found that mosquitoes were less effective at obtaining blood from mice infected with low virulence P. yoelii than from either P. berghei or P. chabaudi infected mice. Plasmodium yoelii is often associated with a more virulent malarial infection in naturally infected rodents (Carter & Walliker 1975, Killick-Kendrick 1978). Day and Edman (1983) suggested that successful transmission of P. yoelii might depend upon the manipulation of host behavior by these associated parasites. Transmission of P. yoelii might be favored if the host were concurrently infected with any other pathogen which modulates host defensive behavior (i.e., not just a more virulent species or strain of Plasmodium). Even if the concomitant pathogen did not directly influence host behavior, more mosquitoes might successfully engorge on dually infected hosts if the presence of a second parasite resulted in an enhanced malarial infection (as shown in Chapters 2, 3, and 4).

Core body temperature, host activity patterns, and mosquito engorgement success were used to assay the effect of P. yoelii and L. mexicana on host health. On the basis of these criteria, P. yoelii and L. mexicana proved to be relatively benign parasites of laboratory mice. Neither parasite markedly affected core body temperature, although mice infected with L. mexicana became slightly hyperthermic by the conclusion of the experiment. This is in contrast to the slight hypothermia observed in mice infected with P. yoelii. In no instance did the hypothermia in these mice reach the low level recorded in mice infected with P. berghei or P. chabaudi (Day & Edman 1984).

Mice infected with L. mexicana remained as active as uninfected mice for the duration of the experiment. Mice infected with P. yoelii remained as active as uninfected mice for several days following parasite inoculation. A drop in daily activity coincided with an increase in parasitemia levels. The clearance of parasites from the peripheral circulatory system was associated with a rapid increase in daily activity. These alterations to daily activity were relatively small compared to the effects produced by P. berghei and P. chabaudi in laboratory mice (Day 1981).

Although core body temperature and host activity can be used to assess host health, vector feeding success provides a more meaningful measure of epidemiologically significant changes in host health. Without blood uptake, subsequent transmission to a susceptible host cannot occur. Although A. aegypti is not a vector of either P. yoelii or L. mexicana; however, it served to bioassay changes in host

anti-vector behavior. Mosquitoes were unable to obtain blood from either uninfected mice or from mice infected with L. mexicana. A few mosquitoes were able to engorge on mice infected with P. yoelii. Successful feeding only occurred for several days during peak parasitemia. Whether the number of mosquitoes which obtained blood from laboratory mice infected with P. yoelii would be sufficient to maintain transmission of the disease under natural conditions is not known. Nor is it known if similar blood-feeding feeding patterns occur in nature. Little evidence is available to indicate what proportion of mosquitoes must obtain blood in order for disease transmission to continue. However, under normal field conditions, only a fraction of all mosquitoes which engorge will survive long enough to transmit the parasite (Bruce-Chwatt 1985). Even if mosquitoes survive the sporogonic incubation period, not all mosquitoes will feed on susceptible hosts, nor will all cases of mosquito feeding result in infection. It is intuitive that the larger the number of mosquitoes which successfully engorge, the larger the number which will survive to develop salivary gland infection. It is only these mosquitoes which are capable of parasite transmission (MacDonald 1957).

Concurrent infection with P. yoelii and L. mexicana affected all parameters which were examined. Malarial infections in dually infected mice were much more severe than in mice infected with P. yoelii only. The progression of L. mexicana was identical in both groups of mice infected with that parasite. Alteration of the various parameters which were examined in this experiment appeared to

result from the abnormally severe malarial infection, and not from any variation in the progression of L. mexicana. For example, mice infected with P. yoelii became hypothermic and mice infected with L. mexicana became hyperthermic. All mice concurrently infected with both parasites became severely hypothermic, not hyperthermic. Daily activity patterns were also affected, with dually infected mice becoming less active.

Profound pathological changes occur during malarial infections. Erythrocyte destruction is accompanied by anemia and release of soluble substances into circulation. Endogenous pyrogen is released from leukocytes, resulting in release of prostaglandins and monoamines (Bruce-Chwatt 1985). Parasitized cells develop "knobs" on their surface which interfere with circulation in the capillaries. Resulting oxygen depletion can lead to lesion development in various organs (Bruce-Chwatt 1985). Conversely, pathology resulting from cutaneous leishmaniasis is primarily restricted to the site of lesion development (Molyneux & Ashford 1983). Inoculation of promastigotes by sand flies is followed by initial invasion of polymorphonuclear leuckocytes to the site of infection. Ulceration results from a combination of cellular infiltration, edema, and vasculitis leading to disruption of blood supply and consequent necrosis. Blood stream dissemination only occurs in rare instances (Marsden & Jones 1985).

It is evident that malaria results in a systemic perturbation of host physiological function (Killick-Kendrick & Peters 1978), whereas cutaneous leishmaniasis affects localized tissue. Mice which were infected with both P. yoelii and L. mexicana developed

abnormally high malarial infections. It is not surprising that modification of host defensive behavior occurred during this period of heightened P. yoelii parasitemia.

Although the model used to test the role of dual infection towards vector feeding success is artificial (i.e., L. mexicana is found in South and Central America while P. yoelii is distributed in central Africa), the results obtained in this study may be suggestive of events in other epidemiological situations. Numerous studies have shown that disease enhancement can result from concomitant parasitic infections (Killick-Kendrick & Peters 1978). Modification of host behavior during concomitant infection would presumably promote vector feeding success in all instances where the host utilizes defensive behavior to prevent blood uptake.

Chapter VIII

FEEDING BEHAVIOR OF *LUTZOMYIA LONGIPALPIS* ON MICE INFECTED WITH *LEISHMANIA MEXICANA AMAZONENSIS*

Introduction

Leishmania mexicana is a major cause of cutaneous leishmaniasis in South and Central America (Lainson & Shaw 1972). Infection in the vertebrate host is initiated through the inoculation of promastigotes by sand flies. The amastigote forms reside in macrophages and are normally localized within discrete cutaneous nodules (Molyneux & Ashford 1983), although dissemination of the parasites may occur (Perez et al. 1979). Sand flies become infected with *L. mexicana* by feeding on areas rich in parasites. Selective feeding on cutaneous lesions would presumably facilitate transmission of *L. mexicana*, although the role of feeding site selection in infection of the vector has not been examined.

Numerous studies have shown that host "defensive" behavior can prevent mosquitoes from successfully feeding on birds (Edman & Kale 1971) and small mammals (Day & Edman 1984, Klowden & Lea 1979, Waage & Nondo 1982, Walker & Edman 1986). Day and Edman (1983) demonstrated that mice infected with malaria exhibited decreased anti-vector defensive behavior when gametocytes were most prevalent. This

behavioral manipulation would favor transmission of the parasite. Research on the role of host defensive behavior toward vector feeding success has thus far been limited to mosquitoes (Day & Edman 1984, Edman & Kale 1971, Klowden & Lea 1979, Waage & Nondo 1982, Walker & Edman 1986) and Reduviid bugs (Schofield 1985, Schofield & White 1982, Schofield et al. 1986). The effect of host behavior on sand fly feeding success is not known, nor is the effect of Leishmania on host behavior.

This study sought to determine whether sand flies preferentially fed on cutaneous lesions of mice infected with L. mexicana. The role of host behavior on the feeding success of L. longipalpis on uninfected mice and on mice infected with L. mexicana was also determined.

Materials and Methods

Animals

Female BALB/c mice were obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts. Mice weighed 25-30 grams and were 14-16 weeks old when used for experiments.

Leishmaniasis

Leishmania mexicana amazonensis (Walter Reed strain 227) was used. The source and history of this isolate have been described (Nolan et al. 1984). Eight-week old experimental animals were infected, using procedures previously described, with amastigotes

obtained from donor mice. Mice were used for experiments 6-8 wks after parasite inoculation.

Sand flies

Lutzomyia longipalpis (Lutz & Neiva) were reared using the techniques of Modi and Tesh (1983). Five-day old *L. longipalpis* were aspirated out of adult holding cages and groups of 10 female flies placed in 30x30x30 cm plexiglass observation chambers. Flies were provided with cotton pledgets soaked with a 30% fructose solution. Fructose was removed after 24 hrs and only water provided. Experiments commenced 24 hrs later, when the flies were 7 days old.

Observations

Individual mice were anesthetized with Nembutal and laid ventral surface down in the center of a cage containing 10 female *L. longipalpis*. The front feet of the mice were tucked under the body, effectively preventing sand flies from feeding on these sites. Potential feeding sites therefore included: left and right hind feet, tail, eyelids (area of exposed skin around the eye), ears, and nose. The total surface area of each of these sites was calculated (by removing the skin from each site and spreading it over a grid) for uninfected mice (UIM) and infected mice (IM). All observations were made in late morning and early afternoon under fluorescent light. Statistical analyses followed methods of Snedecor and Cochran (1967).

Feeding site location. Twenty IM and 10 UIM were individually exposed to groups of 10 *L. longipalpis* for 2 hrs. Locations where individual flies successfully imbibed blood were recorded.

Relationship of landing, probing and blood uptake. The feeding behavior of individual L. longipalpis was observed on UIM and on IM. Observations commenced with initial landing of the fly on the host and were continued until the flies left the host. Foraging and probing sites were recorded, as were locations where blood uptake occurred. Foraging sites were considered to be those areas of exposed skin where individual flies spent periods of 1 sec or more. Probing sites consisted of areas where the mouthparts were actually inserted into the skin, with or without blood uptake. Mice were exposed to L. longipalpis for 2 hr periods. Twenty replicates (200 flies) were observed on UIM and 40 replicates (400 flies) on IM.

Duration of feeding. The length of time required by individual L. longipalpis to feed to repletion on each area of exposed skin on IM and UIM was determined. Feeding duration was defined as the interval between the insertion of mouthparts into the skin of the host and the withdrawal of mouthparts following blood uptake. Feeding bouts in which flies which withdrew the mouthparts prior to the appearance of blood in the abdomen were not considered. Feeding duration was recorded for 25 flies on UIM and 25 flies on IM.

Role of host behavior towards sand fly feeding success. Groups of 10, 20, 30, 40, 50, or 60 female flies were placed in holding cages without water or fructose. Experiments commenced 48 hours later with the introduction of either an unrestrained or a restrained BALB/c mouse (uninfected or infected with L. mexicana) into the holding cage. Restrained mice were anesthetized with Nembutal and laid ventral surface down in the center of the cage. Remaining L.

longipalpis were aspirated from the cage after 1 hour and the number of blood fed flies recorded. Experiments were replicated 17 times on uninfected mice and 8 times on infected mice.

Results

Feeding site location

Fifty flies were observed feeding on UIM and 127 on IM (Table 1). Flies successfully fed on all areas of exposed skin on both UIM and IM; however, the selection of feeding sites was significantly affected by the disease status of the host (Table 1). Flies exposed to UIM showed no clear preference for particular feeding sites, while flies feeding on IM fed predominantly on the infected foot.

Relationship of landing, probing and blood uptake

Fifty-six bouts were observed on UIM (Table 2) and 131 on IM (Table 3). Landing by L. longipalpis was significantly correlated with the total surface area of each site on both UIM ($r=+0.96$) and IM ($r=+0.92$). The high number of flies which landed on the infected foot of IM was not greater than expected based on the total surface area of the site (Chi-Square Goodness of Fit, $P < 0.01$). This suggested that the selection of landing sites occurred randomly.

A small portion of all landings on UIM resulted in probing (Table 2); however, a greater percentage of flies probed on the eyes of UIM mice than expected (Table 4). Probing was much more frequent on IM than expected (based on an equal probability of probing at all sites). The higher than expected incidence of probing on IM resulted

from preferential probing on the infected foot (Tables 3 & 4). A corresponding decrease in the frequency of probing on the tail and on the uninfected foot of the IM occurred. Comparison of probing on UIM with probing on IM demonstrated that observed differences in probing behavior between groups were due almost entirely to this increase in the incidence of probing on the infected foot of IM (Table 5).

Blood uptake occurred in 66.6% (8/12) of probes on UIM and 62.2% (28/45) of probes on IM (Tables 2-4). The uptake of blood was not significantly different on the various sites on a mouse except for a lower than expected frequency on the ears of IM), nor between UIM and IM (Tables 4 - 6).

Duration of feeding

The time required to feed to repletion by L. longipalpis ranged from 208 to 888 sec on UIM and 165 to 1669 sec on IM. The mean time required to feed to repletion on UIM and on IM did not differ greatly (Table 7). The length of time required to feed to repletion on particular sites varied considerably (Table 7).

Role of host behavior towards sand fly feeding success

Sand flies successfully fed on anesthetized mice, but were unable to feed on unrestrained animals (Tables 8-9). The presence of L. mexicana did not affect feeding success on either unrestrained or restrained mice (Tables 8-9).

Table 1. Feeding site selection of female Lutzomyia longipalpis on uninfected mice and on mice infected with Leishmania mexicana amazonensis.

	Uninfected Mice		Infected Mice		
Site	No. Fed	% of Total	No. Fed	% of Total	X^2 Test[a]
Ear	11	22	11	8.7	5.86 *
Eye	13	26	22	17.3	1.72 ns
Nose	2	4	3	2.4	0.35 ns
Tail	13	26	11	8.7	9.20 *
Lft. Ft.	6	12	8	6.2	0.16 ns
Rt. Ft.[b]	5	10	72	56.7	31.82 ****
Totals -	50	100	127	100.0	

[a] - Significant values indicate that the percentage of flies feeding on specific sites on uninfected mice was different than the percentage of flies feeding on the same site on infected mice (X^2 test for equality of 2 multinomials).

[b] - Site of inoculation of L. mexicana in infected mice.

* - $P < 0.05$; **** - $P < 0.00001$; ns - not significant.

Table 2. Blood uptake by Lutzomyia longipalpis in relation to landing and to areas where probing occurred on uninfected mice.

Site	Surface Area (cm^2)	No. of Flies Landing (%[a])	No. of Probes (%[b])	No. Blood Fed (%[c])
Ear	6.1	12 (21.4)	2 (16.6)	1 (8.3)
Eye	0.4	8 (14.2)	5 (62.5)	3 (37.5)
Nose	0.2	7 (12.5)	1 (14.2)	1 (14.2)
Tail	6.4	12 (21.4)	2 (16.6)	1 (8.3)
Lft. Ft.	2.7	8 (14.2)	1 (12.5)	1 (12.5)
Rt. Ft.[d]	2.7	9 (16.1)	1 (11.1)	1 (11.1)
Totals -	18.5	56 -----	12 (21.4)	8 (14.2)

[a] - Percent of total landings on each site.

[b] - Percent of landings on each site which resulted in probing.

[c] - Percent of landings on each site which resulted in blood uptake.

[d] - Corresponds to inoculation site of L. mexicana in infected mice.

Table 3. Blood uptake by Lutzomyia longipalpis in relation to landing sites and to areas where probing occurred on mice infected with Leishmania mexicana amazonensis.

Site	Surface Area (cm^2)	No. of Flies Landing (%[a])	No. of Probes (%[b])	No. Blood Fed (%[c])
Ear	6.1	23 (17.5)	6 (26.0)	1 (4.3)
Eye	0.4	12 (9.1)	6 (50.0)	5 (41.6)
Nose	0.2	11 (8.3)	2 (18.1)	1 (9.0)
Tail	6.4	34 (25.9)	5 (14.7)	2 (5.8)
Lft. Ft.	2.7	16 (12.2)	2 (12.5)	1 (6.2)
Rt. Ft.[d]	6.1	35 (26.7)	24 (68.5)	18 (51.4)
Totals -	21.9	131	45 (34.3)	28 (21.3)

[a] - Percent of total landings on each site.

[b] - Percent of landings on each site which resulted in probing.

[c] - Percent of landings on each site which resulted in blood uptake.

[d] - Site of inoculation of L. mexicana.

Table 4. The conditional probability of probing (given landing) by Lutzomyia longipalpis on uninfected mice and on mice infected with Leishmania mexicana amazonensis.

Feeding Site	Uninfected Mice				Infected Mice			
	Probe	No Probe	X^2 Test[a]		Probe	No Probe	X^2 Test[a]	
Ear	2	10	0.20	ns	6	17	0.49	ns
Eye	5	3	9.35	*	6	6	1.43	ns
Nose	1	6	0.24	ns	2	9	1.51	ns
Tail	2	10	0.20	ns	5	29	7.85	*
Lft. Ft.	1	7	0.44	ns	2	14	3.85	*
Rt. Ft.[b]	1	8	0.67	ns	24	11	24.80	****
Totals -	12	44			45	86		

[a] - Significant values indicate that the incidence of probing was different than expected based on an equal probability of probing at each site (X^2 Test for equality of 2 multinomials).

[b] - Site of inoculation of L. mexicana in infected mice.

* - $P < 0.05$; **** - $P < 0.00001$; ns - not significant.

Table 5. The conditional probability of blood uptake (given probing) by Lutzomyia longipalpis on uninfected mice and on mice infected with Leishmania mexicana amazonensis.

	Uninfected Mice				Infected Mice			
Site	Blood	No Blood	X^2	Test[a]	Blood	No Blood	X^2	Test[a]
Ear	1	1	0.13	ns	1	5	6.11	*
Eye	3	2	0.008	ns	5	1	1.31	ns
Nose	1	0	0.10	ns	1	1	0.13	ns
Tail	1	1	0.13	ns	2	3	1.18	ns
Lft. Ft.	1	0	0.10	ns	1	1	0.13	ns
Rt. Ft.[b]	1	0	0.13	ns	18	6	3.57	ns
Totals -	8	4			28	17		

[a] - Significant values indicate that the incidence of blood uptake was different than expected based on an equal probability of blood uptake at each site (X^2 Test for equality of 2 multinomials).

[b] - Site of inoculation of L. mexicana in infected mice.

* - $P < 0.05$; **** - $P < 0.00001$; ns - not significant.

Table 6. A Comparison of the probability of probing (given landing) or the probability of blood uptake (given probing) on uninfected mice with the probability of probing or the probability of blood uptake on mice infected with Leishmania mexicana amazonensis.

Site	X^2 Value[ab] Probing vs. Not Probing	Blood Uptake vs. No Blood Uptake
Ear	0.44 ns	0.75 ns
Eye	0.31 ns	0.76 ns
Nose	0.04 ns	2.00 ns
Tail	0.02 ns	0.05 ns
Lft. Ft.	0.00 ns	2.00 ns
Rt. Ft.[c]	19.27 *	0.47 ns
Totals -	20.10 *	0.01 ns

[a] - X^2 for equality of proportions.

[b] - Significant values indicate that probing or blood uptake on infected mice was different than probing or blood uptake on uninfected mice.

[c] - Site of inoculation of L. mexicana in infected mice.

* - $P < 0.05$; ns - Not significant

Table 7. Duration of feeding of Lutzomyia longipalpis on uninfected mice and on mice infected with Leishmania mexicana amazonensis.

	Duration of Feeding			
	On Uninfected Mice		On Infected Mice	
Site	No. Fed	Mean Time in secs to Feed (+/-SE)	No. Fed	Mean Time in secs to Feed (+/-SE)
Ear	7	391 (52.5)	1	331 -
Eye	4	643 (66.4)	5	474 (116.2)
Nose	-	- -	1	165 -
Tail	6	575 (52.8)	1	749 -
Lft. Ft.	4	442 (117.0)	4	779 (166.7)
Rt. Ft.[a]	4	469 (143.6)	13	573 (105.8)
Totals -	25	488 (38.2)	25	569 (68.4)

[a] - Site of inoculation of Leishmania mexicana in infected mice.

Table 8. Comparison of the feeding success of Lutzomyia longipalpis on restrained mice and on unrestrained uninfected mice.

Host Condition	Sand fly Group Size	No. of Tests	No. of Flies Blood Fed (%)
Restrained	0-10	2	3/ 15 (20.00)
	11-20	10	91/177 (51.41)
	21-30	3	42/ 74 (56.75)
	31-40	0	- -
	41-50	1	25/ 42 (59.52)
	51-60	1	26/ 55 (47.27)
	Total	17	187/363 (51.51)
Unrestrained	0-10	2	0/ 17 (0.00)
	11-20	10	2/175 (1.14)
	21-30	2	1/ 75 (1.33)
	31-40	2	0/ 75 (0.00)
	41-50	0	- -
	51-60	0	- -
	Total	17	3/342 (0.87)

Table 9. Comparison of the feeding success of Lutzomyia longipalpis on restrained mice and on unrestrained mice infected with Leishmania mexicana amazonensis.

Host Condition	Sand fly Group Size	No. of Tests	No. of Flies Blood Fed (%)
Restrained	0-10	0	- -
	11-20	6	58/108 (53.70)
	21-30	2	23/ 48 (47.91)
	Total	8	81/156 (51.92)
Unrestrained	0-10	0	- -
	11-20	7	2/125 (1.60)
	21-30	1	0/ 22 (0.00)
	Total	17	2/147 (1.36)

Discussion

Feeding site location

Lutzomyia longipalpis fed randomly on UIM in this study. The feeding behavior of this fly appears to be similar to that of mosquitoes, which land and forage indiscriminately on small rodents and racoons (Magnarelli 1979, Walker & Edman 1985). This is in contrast to the specific landing site selection shown by some Tabanidae on cattle (Hollander & Wright 1980, Magnarelli & Anderson 1980, Mullens & Gerhardt 1979). When L. longipalpis fed on mice infected with L. mexicana they preferentially fed on the cutaneous lesions of the animals. No attempt was made to determine if the selection of specific feeding sites resulted from differential landing, probing or blood uptake rates, nor were actual infection rates determined. In spite of this, selective feeding on these parasite-rich areas by L. longipalpis could clearly result in increased vector infection rates.

Certain sand flies have limited access to blood in normal human skin, due to the shortness of the labrum compared to the thickness of the epidermis (Lewis 1987). In patients with post kala-azar dermal leishmaniasis, parasites are often concentrated in cutaneous nodules (Sen Gupta 1964, Indian Council of Medical Research 1980). The epidermis in these nodules is greatly effaced, with the blood vessels dilated in the superficial dermis (Sen Gupta & Bhattachargee 1953). Lewis (1987) suggested that Phlebotomus argentipes should be able to locate blood, and hence parasites, more easily from these nodules than from normal skin.

BALB/c mice infected with L. mexicana develop lesions in which the epidermis is greatly distended. Increased feeding on these lesions by L. longipalpis could have resulted from: 1) an increase in landing on the lesion (compared to the uninfected foot) due to the increased surface area of the lesion, 2) an increase in the attractiveness of the lesion for L. longipalpis, 3) selective probing on the lesion once flies had landed, or 4) more successful blood uptake on the lesion. These possibilities were examined in greater detail in subsequent experiments.

Relationship of landing, probing and blood uptake

The increased blood uptake by L. longipalpis on IM resulted entirely from differences in the probing behavior of the flies on these mice. Lutzomyia longipalpis exhibited no clear preference for landing on lesions, nor was uptake of blood more efficient at these sites once probing was initiated. Probing occurred more frequently on the infected foot of IM than on all other areas, with the exception of the eyes of UIM and IM.

Numerous studies have shown that the manipulation of host-vector interactions by parasites can facilitate pathogen transmission (Molyneux & Jefferies 1986). Enhanced disease transmission can result from changes in the probing ability of infected insects (Anez & East 1984, Beach et al. 1985, Jenni et al. 1980, Rossignol et al. 1984), from increased blood finding success of arthropods on parasitemic hosts (Rossignol et al. 1985), and from changes in the efficacy of defensive behaviors of infected animals (Day & Edman

1983). Rossignol et al. (1985) found that blood uptake was facilitated in animals infected with Plasmodium chabaudi or Rift Valley fever virus once probing was initiated. We suggest that pathological changes in the epidermis produced during L. mexicana infections may also result in enhanced disease transmission (due to increased feeding on those areas where parasites are usually found). Unlike the report by Rossignol et al. (1985); however, these data suggest that facilitated blood uptake may result from factors that come into play prior to the initiation of feeding. The precise mechanism by which this occurs is not known.

Duration of feeding

Blood-feeding insects face certain risks during host contact (Gillet 1967, Rossignol et al. 1985). The predicted strategy for blood-feeding insects therefore consists of a maximization of nutrient intake rate and a minimization of time required to obtain a blood meal (Daniel & Kingsolver 1983).

In contrast to reports on rapid feeding by mosquitoes (Mellink et al. 1982, Walker & Edman 1986) and by the sand fly, Lutzomyia orestes (Mendoza et al. 1983), L. longipalpis fed extremely slowly on both IM and UIM. Based on feeding speed alone, blood feeding by this fly appears to be a relatively inefficient process. A combination of slow feeding by the vector and host defensive behavior may have contributed to the inability of L. longipalpis to feed on both unrestrained UIM and unrestrained IM in these experiments.

Many vertebrates possess behavioral repertoires which limit the duration of host/vector contact (Day & Edman 1984, Edman & Kale 1971, Waage & Nondo 1982, Walker & Edman 1986). Rossignol et al. (1985) suggested that mutualism between parasite and vector could result in a decrease in the duration of feeding, thereby favoring parasite uptake. Unlike P. chabaudi and Rift Valley fever virus (Rossignol et al. 1985), Leishmania mexicana does not appear to affect vector feeding speed.

Role of host behavior towards sand fly feeding success

Day and Edman (1984) found that laboratory mice were highly defensive towards Aedes aegypti, Anopheles quadrimaculatus, Culex nigripalpis, and Culex quinquefasciatus. Unrestrained mice prevented mosquitoes from successfully feeding in most instances. We have found that unrestrained mice were equally as defensive towards L. longipalpis, allowing only a small portion of all flies to feed. This is the first demonstration that the blood feeding success of Diptera other than mosquitoes is affected by host defensive responses. Infection with L. mexicana did not affect host defensive behavior towards sand flies. Although L. mexicana infections in BALB/c mice appear debilitating, transmission is not favored by a manipulation of host behavior.

Feeding Behavior of Lutzomyia longipalpis on mice

Transmission of arthropod-borne diseases depends on the successful uptake of pathogens by a vector. When parasites are localized in discrete areas, as with New World cutaneous leishmaniasis,

preferential selection of feeding sites could result in increased vector infection rates. Pathogens which are systemically distributed presumably would not be affected by feeding site selection. Analysis of data suggests that factors associated with L. mexicana infections in BALB/c mice contribute to feeding on those areas where parasites presumably are most frequent. We have not attempted to determine if preferential feeding site selection would be reflected in actual sand fly infection rates.

Chapter IX

SUMMARY OF CONCLUSIONS

The following conclusions were obtained during this study:

1) BALB/c and C/57 mice infected with Plasmodium yoelii developed an infection that lasted 14-16 days. Peak parasitemia occurred approximately 10 days after parasite inoculation, when 10-15% of all erythrocytes were infected.

2) BALB/c mice infected with Leishmania mexicana amazonensis developed lesions at the site of inoculation. Parasites rapidly disseminated throughout the body of these mice. Lesions developed more rapidly when the infective inocula was increased; however, the outcome of the infection was consistent irregardless of the infective dose.

3) C/57 mice infected with L. mexicana amazonensis developed cutaneous lesions at the site of inoculation. The size of the lesions increased gradually until they reached a maximum diameter 10-15 weeks after parasite inoculation. Gradual healing occurred thereafter. No parasite dissemination was observed.

4) Mice infected with both P. yoelii and L. mexicana developed malarial infections which were more severe than those observed when mice were only infected with P. yoelii. Twenty to 35% of all

erythrocytes were infected during peak P. yoelii parasitemia in these dually infected mice. Infections were enhanced when: i) both parasites were inoculated within 2 days of each other, ii) when L. mexicana was inoculated 3 weeks prior to P. yoelii, and iii) when L. mexicana was inoculated 12 weeks prior to P. yoelii.

5) BALB/c and C/57 mice infected with both P. yoelii and L. mexicana amazonensis developed Leishmania infections which were more severe than those observed when mice were only infected with L. mexicana. Mice infected with both P. yoelii and L. mexicana developed lesions which were significantly larger than those in control mice when: i) both parasites were inoculated within 2 days of each other, ii) when L. mexicana was inoculated 3 weeks prior to P. yoelii, iii) when P. yoelii was inoculated 3 weeks prior to L. mexicana, and iv) when P. yoelii was inoculated 12 weeks prior to L. mexicana (C/57 mice).

6) Several C/57 mice which were infected with both P. yoelii and L. mexicana developed disseminated lesions which did not heal spontaneously. As previously stated, such dissemination was never observed in mice only infected with L. mexicana.

7) Infection with Plasmodium or Leishmania is associated with a well documented suppression of immune function. The enhanced parasitemia that resulted from infection with both P. yoelii and L. mexicana presumably results from these immunosuppressive effects. Drugs which affect immune function could theoretically be used to treat these infections. Agents which modulate T

suppressor cell function were used in an attempt to treat L. mexicana infections in BALB/c and C/57 mice. Cimetidine and 2'-deoxyguanosine were as effective as pentostam (an agent with known anti-Leishmanial activity) at controlling lesion development in these mice. Varying the infective dose used to initiate infections affected the capacity of these drugs to treat clinical symptoms. Further research on the capacity of immune modulation for the treatment of leishmaniasis is warranted.

8) Treatment with pentostam resulted in more severe malaria in mice only infected with P. yoelii and in mice infected with both P. yoelii and L. mexicana.

9) Cimetidine limited the development of L. mexicana in mice infected with this parasite alone and in mice concurrently infected with P. yoelii. Cimetidine did not affect the development of P. yoelii in mice only infected with that parasite; however, P. yoelii infection was markedly limited in mice concurrently infected with L. mexicana. This indicated that manipulation of one infection (L. mexicana) effectively limited the development of a concomitant pathogen (P. yoelii). Such control presumably resulted from the immuno-modulatory effects of cimetidine.

10) BALB/c mice were extremely efficient at preventing blood seeking mosquitoes (Aedes aegypti) from feeding.

11) Mosquitoes which were exposed to mice infected with P. yoelii were only able to engorge during a 3-4 day period. This increase in feeding success was associated with the period of

maximum parasitemia. Very few mosquitoes ever obtained blood from uninfected mice or from mice infected with L. mexicana.

12) Significantly more mosquitoes engorged on mice infected with both P. yoelii and L. mexicana than on uninfected mice, on mice only infected with L. mexicana, or on mice only infected with P. yoelii.

13) Uninfected mice maintained a core body temperature of approximately 36.5-37°C Mice infected with L. mexicana became hyperthermic by the conclusion of the experiment, with the mean daily temperature rising gradually to 38°C. Mice infected with P. yoelii or with both P. yoelii and L. mexicana became hypothermic during the crisis phase of P. yoelii infection. The mean core body temperature of mice infected with P. yoelii or dropped to 36.2°C, while that of mice infected with both P. yoelii and L. mexicana dropped to 34.1°C.

14) The daily activity of uninfected mice and L. mexicana infected mice remained constant over the course of the experiment (approximately 5,000 revolutions per day on each activity wheel). Mice infected with P. yoelii or with both P. yoelii and L. mexicana were less active during peak malarial infection than at other times.

15) Sand flies (Lutzomyia longipalpis) were unable to feed on unrestrained, uninfected mice or on unrestrained, L. mexicana infected mice. Sand fly engorgement was prevented by defensive movements of these animals.

16) Lutzomyia longipalpis were able to feed on restrained mice. Flies preferentially fed on the cutaneous lesions of mice infected with L. mexicana. This preference resulted from selective probing on the lesions, and not from a non-random choice of landing site nor from facilitated blood uptake once probing had commenced.

Outbreaks of leishmaniasis have historically followed epidemics of malaria in certain geographical areas (Pampiglione et al. 1974). This temporal coincidence presumably results from the increased production of mosquitoes and sand flies during periods of favorable environmental conditions (Pampiglione et al. 1974). The importance of vector abundance towards the epidemiology of these diseases was highlighted during the World Health Organization's malaria eradication campaign. Following the widespread application of DDT during the campaign, the incidence of leishmaniasis (Corradetti 1952, Hertig 1949, Lysenko 1971, Nadim & Amini 1970, Seyedi-Rashiti & Nadim 1975) and other Phlebotomus-borne diseases (Hertig 1949, Hadjinicolaou 1958, Tesh & Papaevangelou 1977) dropped markedly in areas where the pesticide was sprayed. This decrease presumably resulted from the elimination of endophilic sand flies by the pesticide.

Although sand fly abundance may be the most important factor contributing to the development of epidemic leishmaniasis, other factors may also be relevant. Pampiglione (1974) suggested that stress such as epidemic malaria or famine would affect the competence of the host immune system. This perturbation would transform an otherwise

inapparent Leishmania infection into a clinically evident case. Evidence has shown that clinically apparent cases of leishmaniasis comprise a small fraction of all infections (Esterre et al. 1987, Fuller et al. 1976 1979, Pampiglione et al. 1974), suggesting that some unknown factor(s) affect the progression of the parasites in the host.

The immunological competence of an organism is crucial to the epidemiology of all infectious diseases. The nutritional state of the host and the presence or absence of concomitant infections are key factors which can modulate immune competence (Harrison et al. 1986, Dowd & Heatley 1984, Killick-Kendrick & Peters 1978). The development of protective immunity to L. mexicana and L. donovoni is clearly affected by protein malnutrition (Harrison et al. 1986, Perez et al. 1984), and there appears to be a reciprocal relationship between undernutrition and the development of visceral leishmaniasis (Harrison et al. 1986). Although Walton et al. (1973) and Pampiglione et al. (1974) suggested that tuberculosis or malaria might affect the course of a Leishmania infection, little research has been directed towards the role concomitant infections might have on the development of the disease.

These results clearly demonstrate the effects concomitant infection with Plasmodium yoelii and Leishmania mexicana amazonensis can have in laboratory mice. Not only were the clinical symptoms of each disease enhanced during dual infections, but factors which affect vector feeding success (and thereby disease transmission) were altered. Although the combination of Leishmania mexicana and

Plasmodium yoelii in BALB/c mice is an artificial system, the results obtained during this study may be applicable to other, more realistic, epidemiological situations.

Perturbation of the host immune system may been responsible for the disease enhancement which occurred in mice infected with P. yoelii and L. mexicana. It seemed plausible that agents which modulate immune function might be effective against Leishmania or Plasmodium infections. We examined the effects of cimetidine, ranitidine, and 2`-deoxyguanosine on the development of leishmaniasis in BALB/c and C/57 mice. Cimetidine and ranitidine inhibit the activity of T suppressor cells by binding to the H_2 histamine receptor (Zapata-Sirvent et al. 1985, Henry et al. 1980), while 2'-deoxyguanosine inhibits the replication of these cells (Bril et al. 1984). Each of these agents limited the development of lesions in BALB/c mice. The control was comparable to, or better, than that obtained using pentostam. However, in no instance did any of these three agents completely eliminate all symptoms of the disease. These data are in agreement with those of Osband et al. (1981) and Gifford et al. (1981), who found that the immuno-restorative effect of cimetidine slowed the development of a given disease rather than eliminating all symptoms. Mucocutaneous and diffuse cutaneous leishmaniasis respond poorly to chemotherapy unless an effective immune response is initiated. Use of agents that activate immune function may have great value for the treatment of these forms of leishmaniasis. Further research on the therapeutic effects of immuno modulating agents against infectious diseases is certainly warranted.

Very few studies have examined the influence that a chemotherapeutic agent aimed at one parasite can have on the development of concomitant pathogens. The system we have been using proved to be an ideal model with which to examine this issue. The results clearly indicated that the agents used to treat one disease can have an effect on the progression of an unrelated pathogen. Pentostam is the drug of choice for the treatment of leishmaniasis. It is fairly effective, cheap, and the side-effects are normally mild (Marsden & Jones 1985). Based on our studies with laboratory mice, caution should be exercised when treating patients who might also have malaria. Mice that were infected with P. yoelii and were treated with pentostam developed more severe infections than control mice. When pentostam was used to treat the Leishmania infection in mice infected with both P. yoelii and L. mexicana, the malaria was less severe than the infection in control mice infected with both parasites. However, this infection was still more severe than that in control mice that were only infected with P. yoelii. Cimetidine proved to be as effective as pentostam at limiting the development of leishmaniasis in BALB/c mice. However, all mice treated with cimetidine (including those concurrently infected with P. yoelii and L. mexicana) developed malarial infections which were less severe than that observed in control animals. These results clearly indicate that prior to the initiation of chemotherapy, considerable care should be taken to determine what concomitant parasites infect a given host. It is evident that chemotherapeutic agents may adversely affect host health in some instances.

Vector-borne diseases depend upon vector contact with an infected host and, following an appropriate extrinsic incubation period, subsequent feeding on a susceptible organism. Previous research has shown that rodents and certain birds use defensive movements to prevent mosquitoes from engorging (Day & Edman 1984b, Edman & Kale 1971, Edman et al. 1972 1974, Walker & Edman 1986). Day & Edman (1983) found that infection with P. berghei or P. chabaudi affected host behavior so that vector feeding success was facilitated. However, infection with P. yoelii did not significantly affect host behavior. Mosquitoes attempting to feed on mice infected with P. yoelii were much less successful at engorging than on mice infected with P. berghei or P. chabaudi (Day & Edman 1983).

We have shown that mice concurrently infected with P. yoelii and L. mexicana develop more severe malaria than that which occurs in mice only infected with P. yoelii. Aedes aegypti were only successful at feeding on these dually infected mice during the crisis phase of the Plasmodium infection. These mosquitoes were unable to engorge on uninfected mice or on mice infected with L. mexicana. Very few A. aegypti were able to feed on mice only infected with P. yoelii. The other parameters which were examined were also significantly affected in mice infected with both parasites. The mean core body temperature and the mean amount of daily activity dropped markedly during the crisis phase of the malarial infection in mice infected with both P. yoelii and L. mexicana. These parameters were also affected in mice only infected with P. yoelii; however, the level of daily activity

and the mean core body temperature decreased to a lesser degree than that observed in dually infected mice.

The behavioral and physiological changes which were noted in dually infected mice resulted from the increased severity of the Plasmodium infection. No comparable changes were noted in any of the other groups of mice, although similar, but less marked, changes occurred in mice only infected with P. yoelii.

It appears that mosquitoes would engorge more frequently on rodents concurrently infected with P. yoelii and a Leishmania sp. than on mice infected with either parasite alone. Malarial transmission would presumably be facilitated by this selective process. The transmission of leishmaniasis might also be favored by concurrent infection with these pathogens, as mice are extremely effective at preventing sand flies from engorging. This possibility remains unanswered; however, as rodents serve as the primary reservoir for many forms of leishmaniasis, this issue is especially relevant.

Cutaneous leishmaniasis is unusual among parasitic diseases in that the pathogens are restricted to localized sites. Vectors presumably become infected by feeding on these parasite rich areas. Lutzomyia longipalpis selectively fed on the lesions of anesthetized L. mexicana infected mice. This preferential selection of feeding site did not result from discrimination during landing. However, once landing had taken place, the flies probed more frequently on lesions than on other sites. The probability of successfully obtaining blood once probing had commenced was equal on all sites. This

selective process presumably facilitates the transmission of leishmaniasis.

LITERATURE CITED

Abdel-Wahab, M.F., Powers, K.C., Mahmoud, S.S. and W.C. Good. 1974. Suppression of schistosome granuloma formation by malaria in mice. American Journal of Tropical Medicine and Hygiene 23, 915-918.

Adler, S. 1954. The behavior of Plasmodium berghei in the golden hamster Mesocricetus auratus infected with visceral leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 48, 431-440.

Adler, S. and Ber, M. 1941. The transmission of Leishmania tropica by the bite of Phlebotomus papatasi. Indian Journal of Medical Research 29, 803-909.

Aikawa, M., Suzuki, M., and Gutierrez, Y. 1980. Pathology of malaria. In: Malaria, Volume II (Kreier, J.P., Ed.). Academic Press, New York. Pp. 47-102.

Allen, P.C., Frerichs, W.M., and Holbrook, A.A. 1975. Experimental acute Babesia caballi infections. II. Response of platelets and fibrinogen. Experimental Parasitology 37, 373-379.

Alving, C.R., and Swartz, G.M., Jr. 1984. Preparation of liposomes for use as drug carriers in the treatment of leishmaniasis. In: Liposome Technology, Volume II (Gregoriadis, G., Ed.). CRC Press, Boca Raton. Pp. 55-68.

Anez, N. and J.S. East. 1984. Studies on Trypanosoma rangeli Tejera, 1920. II. Its effect on feeding behavior of Triatomine bugs. Acta Tropica 41, 93-95.

Ayele, T. and Ali, A. 1984. The distribution of visceral leishmaniasis in Ethiopia. American Journal of Tropical Medicine and Hygiene 33, 548-552.

Balashov, Y.S. 1984. Interaction between blood-sucking arthropods and their hosts, and its influence on vector potential. Annual Review of Entomology 29, 137-156.

Barker, L.R. 1971. Experimental malaria: Effects upon the immune response to different antigens. Journal of Infectious Diseases 123, 99-101.

Barral, A., Petersen, E.A., Sacks, D.A., and Neva, F.A. 1983. Late metastatic leishmaniasis in the mouse: A model for mucocutaneous disease. American Journal of Tropical Medicine and Hygiene 32, 277-285.

Beach, R., Kiilu, G., Hendricks, L., Oster, C., and Leeuwenburg, J. 1984. Cutaneous leishmaniasis in Kenya: transmission of Leishmania major to man by the bite of naturally infected Phlebotomus duboscqi. Transactions of the Royal Society of Tropical Medicine and Hygiene 78, 747-751.

Beach, R., Kiilu, G., and Leeuwenburg, J. 1985. Modification of sand fly biting behavior by Leishmania leads to increased parasite transmission. American Journal of Tropical Medicine and Hygiene 34, 279-283.

Belehu, A., Louis, J.A., Pugin, P. and Miescher, P.A. 1980. Immunopathological aspects of leishmaniasis. Springer Seminar on Immunopathology 2, 399-415.

Bender, E.M., Hansbrough, J.F., Whitefield, J., Anderson, J., and Claman, H.N. 1984. Prevention of postburn alterations in helper and suppressor T lymphocytes by cimetidine. Surgical Forum 35, 156.

Berman, J.D. 1981. Activity of imidazoles against Leishmania tropica in human macrophage cultures. American Journal of Tropical Medicine and Hygiene 30, 566-569.

Berman, J.D. 1983. Leishmaniasis. In: Leishmaniasis In Current Therapy (Conn, O.O., Ed.). W.B. Saunders, Philadelphia. Pp. 27-29.

Berman, J.D. 1985. Experimental chemotherapy of leishmaniasis - a critical review. In: Leishmaniasis (Chang, K.-P., and Bray, R.S., Eds.). Elsevier, New York. Pp. 111-138.

Bettini, S., Maroli, M., and Gradoni, L. 1981. Leishmaniasis in Tuscany (Italy): (IV) An analysis of all recorded human cases. Transactions of the Royal Society of Tropical Medicine and Hygiene 75, 338.

Black, C.D.V., Watson, G.J., and Ward, R.J. 1977. The use of pentostam liposomes in the chemotherapy of experimental leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 71, 550-552.

Blackwell, J.M. and Ulczak, O.M. 1984. Immunoregulation of genetically controlled acquired responses to Leishmania donovoni infection in mice: Demonstration and characterization of suppressor T cells in noncure mice. Infection and Immunity 44, 97-102.

Bomford, R. and Wedderburn, N. 1973. Depression of immune response to moloney leukaemia virus by malarial infection. Nature 242, 471-472.

Board on Science and Technology for International Development (BOSTID). 1983. Manpower Needs And Career Opportunities In The Field Aspects Of Vector Biology. National Academy Press, Washington, D.C. 53 pp.

Brabin, B.J. 1983. An analysis of malaria in pregnancy in Africa. Bulletin of the World Health Organization 61, 1005-1016.

Bray, R.S. and Anderson, M.J. 1979. Falciparum malaria and pregnancy. Transactions of the Royal Society of Tropical Medicine and Hygiene 73, 427-431.

Bril, H., Van Den Akker, Th.W., Molendijk-Lok, B.D., Bianchi, A.T.J., and Benner, R. 1984. Influence of 2'-deoxyguanosine upon the development of DTH effector T cells and suppressor T cells in vivo. The Journal of Immunology 132, 599-604.

Brogden, R.N., Heel, R.C., Speight, T.M., and Avery, G.S. 1978. Cimetidine: A review of its pharmacological properties and therapeutic efficacy in peptic ulcer disease. Drugs 15, 93-131.

Bruce-Chwatt, L.J. 1970. Global review of malaria control and eradication by attack on the vector. Miscellaneous Publication of the Entomological Society of America 7, 7-23.

Bruce-Chwatt, L.J. 1983. Malaria and Pregnancy. British Medical Journal 286, 1457-1458.

Bruce-Chwatt, L.J. 1985. Essential Malariology. John Wiley and Sons, New York. 452 pp.

Bryceson, A.D.M., Chulay, J.D., Mugambi, M., Were, J.B., Gachichi, G., Chunge, C.N., Muigai, R., Bhatt, S.M., Ho, M., Spencer, H.C., Meme, J., and Anabwani, G. 1985. Visceral leishmaniasis unresponsive to antimonial drugs. II. Response to high dosage sodium stibogluconate or prolonged treatment with pentamidine. Transactions of the Royal Society of Tropical Medicine and Hygiene 79, 705-714.

Bygbjerg, I.C. and Flachs, H. 1986. Effect of chloroquine on human lymphocyte proliferation. Transactions of the Royal Society of Tropical Medicine and Hygiene 80, 231-235.

Carter, R., and Walliker, D. 1975. New observations on the malaria parasites of the central African Republic: Plasmodium vinckei petteri subsp. nov. and Plasmodium chabaudi landau, 1965. Annals of Tropical Medicine and Parasitology 69, 187-196.

Carter, R., and Walliker, D. 1977. The malaria parasites of rodents of the congo (Brazzaville): Plasmodium chabaudi adami subsp. nov. and Plasmodium vinckei lentum Landau, Michel, Adam and Boulard, 1970. Annals de Parasitologie Humaine et Comparee 51, 637-646.

Celada, A., Cruchaud, A., and Perrin, L.H. 1982. Opsonic activity of human immune serum on in vitro phagocytosis of Plasmodium falciparum infected red blood cells by monocytes. Clinical and Experimental Immunology 47, 635-644.

Chance, M.L. 1981. Leishmaniasis. British Medical Journal 283, 1245-1247.

Chang, K.-P., Fong, D., and Bray, R.S. 1985. Biology of Leishmania and leishmaniasis. In: Leishmaniasis (Chang, K.-P. and Bray, R.S., Eds.). Elsevier, New York. Pp. 1-30.

Chung, H.L., Feng, L.-C., and Feng, L.-S. 1951. Observations concerning the successful transmission of kala-azar in North China by the bites of naturally infected Phlebotomus chinensis. Peking Natural History Bulletin 19, 302-326.

Cook, I.F. 1985. Herpes zoster in children following malaria. Journal of Tropical Medicine and Hygiene 88, 261-264.

Corkill, N.L. 1948. The poisonous wild duster yam, Discorea demetorum Pax, as a famine food in the Anglo-Egyptian Sudan. Annals of Tropical Medicine and Parasitology 42, 635-644.

Corradetti, A. 1952. The epidemiology and control of oriental sore in Abruzzo, Italy. American Journal of Tropical Medicine and Hygiene 1, 618-622.

Correa, M., Narayanan, P.R., and Miller, H.C. 1980. Suppressive activity of splenic adherent cells from Plasmodium chabaudi infected mice. Journal of Immunology 125, 749-754.

Coutinho, S.G., Louis, J.A., Mauel, J. and Engers, H.D. 1984. Induction by specific T lymphocytes of intracellular destruction of Leishmania major in infected murine macrophages. Parasite Immunology 6, 157-170.

Daniel, T.L. and Kingsolver, J.G. 1983. Feeding strategy and the mechanics of blood sucking in insects. Journal of Theoretical Biology 105, 661-672.

Davis, C.E. 1982. Thrombocytopenia: A uniform complication of African trypanosomiasis. Acta Tropica 39, 123-127.

Dawkins, R. 1983. The Extended Phenotype. W.H. Freeman, San Fransisco. 307 pp.

Day, J.F. 1981. The effect of host health on mosquito engorgement success and its possible importance in disease transmission. Ph.D. Dissertation, University of Massachusetts, Amherst, MA. 160 pp.

Day, J.F. and Edman, J.D. 1983. Malaria renders mice susceptible to mosquito feeding when gametocytes are most infective. Journal of Parasitology 69, 163-170.

Day, J.F., and Edman, J.D. 1984a. The importance of disease induced changes in mammalian blood temperature to mosquito blood feeding. Comparative Biochemistry and Physiology 77A, 447-452.

Day, J.F. and Edman, J.D. 1984b. Mosquito engorgement on normally defensive hosts depends on host activity patterns. Journal of Medical Entomology 21, 732-740.

Day, J.F., Ebert, K.M., and Edman, J.D. 1983. Feeding patterns of mosquitoes (Diptera: Culicidae) simultaneously exposed to malarious and healthy mice, including a method for separating blood meals from conspecific hosts. Journal of Medical Entomology 20, 120-127.

Dowd, P.S., and Heatley, R.V. 1984. The influence of undernutrition on immunity. Clinical Science 66, 241-248.

Drews, J. 1985. The experimental and clinical use of immunomodulating drugs in the prophylaxis and treatment of infections. In: Non-Antibiotic Prevention And Treatment Of Infectious Diseases (Marget, W., Hanson, L.Aa., Young, L.S., and Weuta, H., Eds.). Infection 13 (supplement 2), S241-S250.

Dye, C. and Hasibeder, G. 1986. Population dynamics of mosquito-borne diseases: effects of flies which bite some people more frequently than others. Transactions of the Royal Society of Tropical Medicine and Hygiene 80, 69-77.

Edman, J.D. and Kale, H.W. 1971. Host behavior: its influence on the feeding success of mosquitoes. Annals of the Entomological Society of America 64, 513-516.

Edman, J.D., Webber, L.A., and Kale II, H.W. 1972. Effect of mosquito density on the interrelationship of host behavior and mosquito feeding success. American Journal of Tropical Medicine and Hygiene 21, 487-491.

Edman, J.D., Webber, L.A., and Schmid, A.A. 1974. Effect of host defenses on the feeding patterns of Culex nigripalpus when offered a choice of blood sources. Journal of Parasitology 60, 874-883.

Esterre, O., Ridel, P.R., Jamet, P., and Dedet, J.P. 1987. Cutaneous factors in susceptibility to American cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 81, 160.

Evered, D., and Whelen, J. (Eds.). 1983. Malaria And The Red Cell. Ciba Foundation Symposium 94. Pitman Books, London.

Ferrante, A. and Goh, D.H.B. 1984. The effect of anti-malarial drugs on human natural killer cells in vitro. Parasite Immunology 6, 571-580.

Freeeman, R.B. 1978. T cell function during fatal and self-limiting malarial infections of mice. Cellular Immunology 41, 373-379.

Fuller, G.K., Lemma, A., Haile, T., and Atwood, C.L. 1976. Kala-azar in Ethiopia. 1: Leishmanin skin test in Setit Humera, a kala-azar endemic area in north-western Ethiopia. Annals of Tropical Medicine and Parasitology 70, 147-163.

Fuller, G.K., Lemma, A., Haile, T., and Atwood, C.L. 1979. Kala-azar in Ethiopia: Survey of southwest Ethiopia. The leishmanin skin test and epidemiological studies. Annals of Tropical Medicine and Parasitology 73, 417-430.

Gardner, I.D. 1980. The effect of aging on susceptibility to infection. Review of Infectious Diseases 2, 801.

Garnham, P.C.C. 1966. Malaria Parasites And Other Haemosporidia. Blackwell Scientific Publications, Oxford. 1,132 pp.

Garnham, P.C.C. 1980. Malaria in its various vertebrate hosts. In: Malaria, Vol. 1 (Kreier, J.P., Ed.). Academic Press, New York. Pp. 96-144.

Gery, I. and Eidinger, D. 1977. Selecting and opposing effects of cytochalasin B and other drugs on lymphocyte responses to different doses of mitogens. Cellular Immunology 30, 147-155.

Gifford, R.R.M., Sr., Hatfield, S.M., and Schmidtke, J.R. 1980. Cimetidine-induced augmentation of human lymphocyte blastogenesis by mitogen, bacterial antigen, and alloantigens. Transplantation 29, 143-148.

Gilles, H.M., Lawson, J.B., Sibelas, M., Voller, A., and Allen, N. 1969. Malaria, anemia, and pregnancy. Annals of Tropical Medicine and Parasitology 63, 245-263.

Gillet, J.D. 1967. Natural selection and feeding speed in a blood-sucking insect. Proceedings of the Royal Society of Biological Sciences 167, 316-329.

Greenwood. B.M. 1974. Immunosuppression in malaria and trypanosomiasis. In: Parasites In The Immunized Host: Mechanisms Of survival. Ciba Foundation Symposium 25. Elsevier, Amsterdam. Pp. 137-146.

Greenwood, B.M. 1971. Immunosuppression in murine malaria. I. General characteristics. Clinical and Experimental Immunology 8, 467-478.

Grimstad, P.R., Ross, Q.E., and Craig, G.B.,Jr. 1980. Aedes triseriatus (Diptera: Culicidae) and La Crosse virus. II. Modification of mosquito feeding behavior by virus infection. Journal of Medical Entomology 17, 1-7.

Gustafson, T.L., Reed, C.M., McGreevy, P.B., Pappas, M.G., Foz, J.C., and Lawyer, P.G. 1985. Human cutaneous leishmaniasis acquired in Texas. American Journal of Tropical Medicine and Hygiene 34, 58-63.

Hadjinicolaou, J. 1958. Present status of Phlebotomus in certain areas of Greece. Bulletin of the World Health Organization 19, 967-979.

Halstead, S.B. 1984. Dengue. In: Tropical And Geographical Medicine (Warnrer, K.S. and Mahmoud, A.A.F., Eds.). McGraw-Hill, New York. Pp. 652-659.

Hanson, W.L. 1981. Chemotherapy and the immune response in protozoal infections. Journal of Protozoology 28, 27-30.

Harinasuta, T., Migascos, S., and Bunnag, D. 1962. Chloroquine resistance in Plasmodium falciparum in Thailand. Unesco First Regional Symposium of Scientific Knowledge of Tropical Parasites, Singapore, 1962. Pp. 148-153.

Harrison, L.H., Naidu, T.G., Drew, J.S., Eduardo de Alcenar, J., and Pearson, R.D. 1986. Reciprocal relationships between undernutrition and the parasitic disease visceral leishmaniasis. Reviews of Infectious Diseases 8, 447-453.

Harwood, R.F. and James, M.T. 1979. Entomology In Human And Animal Health. MacMillan Publishing Co. Inc., New York. 548 pp.

Henry, D.A., MacDonhald, I.A., Kitchingman, G., Bell, G.D., and Langman, M.J.S. 1980. Cimetidine and ranitidine: comparison of effects on depatic drug metabolism. British Medical Journal, 281, 775-777.

Hertig, M. 1949. Phlebotomus and residual DDT in Greece and Italy. American Journal of Tropical Medicine and Hygiene 29, 773-805.

Herzog, C., Kibbler, C.C., Ellis, C.J. and Mtawali, C.V. 1983. Falciparum malaria resistant to chloroquine and fansidar: implications for prophylaxis. British Medical Journal 287, 947-948.

Hill, J.O. 1986. Pathophysiology of experimental leishmaniasis: Pattern of development of metastatic disease in the susceptible host. Infection and Immunity 52, 364-369.

Hodgkinson, V.H. and Herman, R. 1980. In vivo assay of viability of amastigotes of Leishmania donovoni. Journal of Parasitology 66, 245-249.

Hollander, A.L. and Wright, R.E. 1980. Impact of Tabanids on cattle: Blood meal size and preferred feeding sites. Journal of Economic Entomology 73, 431-333.

Howard, J.G., Hale, C., and Chan-Liew, W.L. 1980a. Immunological regulation of experimental cutaneous leishmaniasis. 1: Immunogenetic aspects of susceptibility to Leishmania tropica in mice. Parasite Immunology 2, 303-314.

Howard, J.G., Hale, C., and Chan-Liew, W.L. 1980b. Immunological regulation of experimental cutaneous leishmaniasis. II. Nature and significance of specific suppression of cell-mediated immunity in mice highly susceptible to Leishmania tropica. Journal of Experimental Medicine 152, 594-607.

Hurvitz, D. and Hirschhorn, K. 1965. Suppression of in vitro lymphocyte responses by chloroquine. New England Journal of Medicine 273, 23-26.

Indian Council of Medical Research. 1980. Proceedings Of The Indo-UK Workshop On Leishmaniasis, December 6-10, Patna, New Delhi.

Jaroskova, L., Selim, M.M., Vlasin, Z., and Al-Taqui, M. 1986. Study of T cell subsets in patients with cutaneous leishmaniasis. Parasite Immunology 8, 381-389.

Jefferies, D., Livesey, J.L., and Molyneux, D.H. 1986. Fluid mechanics of bloodmeal uptake by Leishmania-infected sandflies. Acta Tropica 43, 43-53.

Jelliffe, E.F.P. 1968. Low birth-weight and malarial infection of the placenta. Bulletin of the World Health Organization 33, 69-78.

Jenni, L., Molyneux, D.H., Livesey, J.L., and Galun, R. 1980. Feeding behavior of tsetse flies infected with salivarian trypanosomes. Nature, London 283, 383-385.

Juliano, R.L. 1982. Liposomes and the reticuloendethelial system: interactions of liposomes with macrophages and behavior of liposomes in vivo. In: Targeting Of Drugs (Gregoriadis, G., Senior, J., and Trouet, A., Eds.). Plenum Press, New York. Pp. 285.

Kager, P.A., Rees, P.H., Wellde, B.T., Hockmeyer,W.T., and Lyerly, W.H. 1981. Allopurinol in the treatment of visceral leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 75, 556-559.

Khansari, N., Mariangela, S., and Segre, D. 1981. Immunosuppression in murine malaria: A soluble immunosuppressive factor derived from Plasmodium berghei-infected blood. Journal of Immunology 127, 1889-1893.

Killick-Kendrick, R. 1974. Parasitic protozoa of the blood of rodents. II. Haemogregarines, malaria parasites and piroplasms of rodents: an annoted checklist and host index. Acta Tropica 31, 28-69.

Killick-Kendrick, R. 1979. Biology of Leishmania in phlebotomine sandflies. In: Biology Of The Kinetoplastida, Volume 2 (Lumsden, W.H.R. and Evans, D.A., Eds.). Academic Press, New York. Pp. 395-460.

Killick-Kendrick, R. and Molyneux, D.H. 1981. Transmission of leishmaniasis by the bite of phlebotomine sandflies: possible mechanisms. Transactions of the Royal Society of Tropical Medicine and Hygiene 75, 152-154.

Killick-Kendrick, R. and Peters, W. (Eds.) 1978. Rodent Malaria. Academic Press, New York. 406 pp.

Killick-Kendrick, R., Molyneux, D.H. and Ashford, R.W. 1974. Leishmania in phlebotomid sandflies. I. Modifications of the flagellum associated with attachment to the mid-gut and oesophageal valve of the sandfly. Proceedings of the Royal Society of London, Series B 187, 409-419.

Killick-Kendrick, R., Leaney, A.J., Ready, P.D. and Molyneux, D.H. 1977. Leishmania in phlebotomid sandflies. IV. The transmission of Leishmania mexicana amazonensis to hamsters by the bite of experimentally infected Lutzomyia longipalpis. Proceedings of the Royal Society of London, Series B. 196, 105-115.

Killick-Kendrick, R., Leaney, A.J., Peters, W., Rioux, J.-A., and Bray, R.S. 1985. Zoonotic cutaneous leishmaniasis in Saudia Arabia: the incrimination of Phlebotomus papatasi as the vector in the Al-Hassa oasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 79, 252-255.

Khusmith, S., Druilhe, P. and Gentelini, M. 1982. Enhanced Plasmodium falciparum merozoite phagocytosis by monocytes from immune individuals. Infection and Immunity 35, 874-879.

Kinnamon, K.E., Steck, E.A., Loizeaux, P.S., Hanson, W.L., Chapman, W.L., and Waits, V.B. 1978. The antileishmanial activity of lepidines. American Journal of Tropical Medicine and Hygiene 27, 751-757.

Klowden, M. J. and Lea, A.O. 1979. Effect of defensive host behavior on the blood meal size and feeding success of natural populations of mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 15, 514-517.

Krettli, A.U. 1977. Exacerbation of experimental Trypanosoma cruzi infection in mice by concomitant malaria. Journal of Protozoology 24, 514-518.

Lainson, R. and Shaw, J. 1972. Leishmaniasis of the new world: Taxonomic problems. British Medical Bulletin 28, 44-48.

Lainson, R. and Shaw, J. 1973. Leishmanias and leishmaniasis in the New World, with particular reference to Brazil. Bulletin of the Pan American Health Organization 7, 1-19.

Lelchuk, P. and Playfair, J.H.L. 1980. Two distinct types of non-specific immunosuppression in murine malaria. Clinical and Experimental Immunology 42, 428-435.

Lewis, D.J. 1974. The biology of phlebotomidae in relation to leishmaniasis. Annual Review of Entomology 19, 363-384.

Lewis, D.J. 1984. Trophic sensilla of phlebotomine sandflies. Transactions of the Royal Society of Tropical Medicine and Hygiene 78, 416.

Lewis, D.J. 1987. Depth of penetration of vertebrate skin by phlebotomine sandflies (Diptera: Psychodidae). Annals of Tropical Medicine and Parasitology 81, 173-179.

Liew, F.Y., Hale, C., and Howard, J.G. 1982. Immunologic regulation of experimental cutaneous leishmaniasis. V. Characterization of effector and specific suppressor T cells. Journal of Immunology 128, 1917-1922.

Lima, G.C., Engers, H.D., and Louis, J.A. 1984. Adoptive transfer of delayed type hypersensitivity reactions specific for Leishmania major antigens to normal mice using murine T cell populations and clones generated in vitro. Clinical and Experimental Immunology 57, 130.

Loose, L.D., Cook, J.A., and CiLuzio, N.R. 1972. Malarial immunosuppression, a macrophage mediated defect. Proceedings of the Helminthological Society of Washington 39, 484-491.

Lwin, M., Last, C., Targett, G.A.T., and Doenhoff, M.J. 1982. Infection of mice concurrently with Schistosoma mansoni and rodent malarias: Contrasting effects of patent S. mansoni infections on Plasmodium chabaudi, P. yoelii, and P. berghei. Annals of Tropical Medicine and Parasitology 76, 265-273.

Lysenko, A.Ja. 1971. Distribution of leishmaniasis in the Old World. Bulletin of the World Health Organization 44, 515-520.

MacDonald, G. 1957. The Epidemiology And Control Of Malaria. Oxford University Press, London. 201 pp.

Maegraith, B.G. 1977. Interdependence: The pathology of malaria. American Journal of Tropical Medicine and Hygiene 26, 344-354.

Maegraith, B.G. 1981. Aspects of the pathogenesis of malaria. South Asian Journal of Tropical Medicine and Public Health 12, 251-261.

Magnarelli, L.A. 1979. Feeding behavior of mosquitoes (Diptera: Culicidae) on man, Raccoons, and White-footed Mice. Annals of the Entomological Society of America 72, 162-166.

Magnarelli, L.A. and Anderson, J.F. 1980. Feeding behavior of Tabanidae (Diptera) on cattle and serological analyses of partial blood meals. Environmental Entomology 9, 664-667.

Manson-Bahr, P.E.C. 1971. Leishmaniasis. International review of Tropical Medicine 4, 123-140.

Marinkelle, C.J. 1980. The control of leishmaniasis. Bulletin of the World Health Organization 58, 807-818.

Marsden, P.D., Cuba, C.C., Barreto, A.C., Sampaio, R.N., and Rocha, R.A.A. 1979. Nifurtimox in the treatment of South American leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 75, 391-394.

Marsden, P.D. and Jones, T.C. 1985. Clinical manifestations, diagnosis and treatment of leishmaniasis. In: Leishmaniasis (Chang, K.-P., and Bray, R.S., Eds.). Elsevier, New York. Pp. 193-198.

Marsh, M., Bplzau, E., White, J., and Helenius, A. 1983. Interactions of Semliki Forest virus spike glycoprotein rosettes and vesicles with cultured cells. Journal of Cell Biology 96, 455-461.

Martin, S.K., Oduola, A.M.J., and Milhous, W.K. 1987. Reversal of chloroquine resistance in Plasmodium falciparum by verapamil. Science 235, 899-901.

McBride, J.S., and Micklem, H.S. 1977. Immunosuppression in murine malaria. II. The primary response to bovine serum albumin. Immunology 33, 253-259.

McGregor, I.A. 1972. Immunology of malarial infection and its possible consequences. British Medical Bulletin 28, 22.

Mellink, J.J. 1981. Selections for blood-feeding efficiency in colonized Aedes aegypti. Mosquito News 41, 119-125.

Mellink, J.J., Poppi, D.M.C., and Van Duin, G.J.T. 1982. Factors affecting the blood-feeding process of laboratory strain of Aedes aegypti on rodents. Entomologique Experementalie & Applique 31, 229-238.

Mendoza, J.L., Gonzalez, O.F., Rodriguez, M.C., and Navarro, A. 1983. Estudio de la actividad hematofagica y el tiempo de ingesta de Lutzomyia (C) orestes (Diptera, psychodidae). Informe preliminar. Revista Cubana de Medicina Tropical 35, 257-262.

Miller, L.H., Powers, K.G., Shiroishi, T. 1977. Plasmodium knowlesi: Functional immunity and anti-merozoite antibodies in rhesus monkeys after repeated infection. Experimental Parasitology 41, 105-111.

Modi, G.B. and Tesh, R.B. 1983. A simple technique for mass rearing Lutzomyia longipalpis and Phlebotomus papatasi (Diptera: Psychodidae) in the laboratory. Journal of Medical Entomology 20, 568-569.

Molyneux, D.H., and Ashford, R.W. 1983. The Biology Of Trypanosoma And Leishmania, Parasites Of Man And Domestic Animals. Taylor and Francis, London. 294 pp.

Molyneux, D.H. and Jefferies, D. 1986. Feeding behavior of pathogen-infected vectors. Parasitology 92, 721-736.

Moore, D.V. and Lanier, J.E. 1961. Observations on two Plasmodium falciparum infections with an abnormal response to chloroquine. American Journal of Tropical Medicine and Hygiene 10, 5-9.

Morges, W., and Weidanz, W.P. 1980. Plasmodium yoelii: The thymus-dependent lymphocyte in mice immunodepressed by malaria. Experimental Parasitology 50, 188-194.

Mullens, B.A. and Gerhardt, R.R. 1979. Feeding behavior of some Tennessee Tabanidae. Environmental Entomology 8, 1047-1051.

Murray, H.W., Carriero, S.M., and Donelly, D.M. 1986. Presence of a macrophage-mediated suppressor cell mechanism during cell-mediated immune response in experimental visceral leishmaniasis. Infection and Immunity 54, 487-493.

Nadim, A., and Amini, H. 1970. The effect of antimalaria spraying on the transmission of zoonotic cutaneous leishmaniasis. Tropical and Geographical Medicine 22, 479-481.

Naik, S.R., Rao, P.N., Datta, D.V., Mehta, S.K., Mahajan, R.C., Mehta, S., and Chhuttani, P.N. 1979. Transactions of the Royal Society of Tropical Medicine and Hygiene 73, 61-65.

Neal, R.A. 1976. Effect of sodium stibogluconate and pyrimethamine on mouse infections with Leishmania mexicana. Annals of Tropical Medicine and Parasitology 70, 252.

Neal, R.A. and Hale, C. 1983. A comparative study of susceptibility of inbred and outbred mouse strains compared with hamsters to infection with new world cutaneous leishmaniases. Parasitology 87, 7-13.

New, R.R.C., Chance, M.L., Thomas, S.C., and Peters, W. 1978. Anti-leishmanial activity of antimonials entrapped in liposomes. Nature 272, 55-56.

Nickol, A.D. and Bonventre, P.F. 1985a. Visceral leishmaniasis in congenic mice of susceptible and resistant phenotypes: Immunosuppression by adherent spleen cells. Infection and Immunity 50, 160-168.

Nickol, A.D. and Bonventre, P.F. 1985b. Visceral leishmaniasis in congenic mice of susceptible and resistant phenotypes: T-lymphocyte-mediated immunosuppression. Infection and Immunity 50, 169-174.

Nickol, A.D. and Bonventre, P.F. 1985c. Visceral leishmaniasis in congenic mice of susceptible and resistant phenotypes: Immunosuppression by adherent spleen cells. Infection and Immunity 50, 160-168.

Nolan, L.L., Berman, J.D., and Giri, L. 1984. The effect of Formycin B on mRNA translation and uptake of purine precursors in Leishmania mexicana. Biochemistry International 9, 207-218.

Osband, M.E., Shen, Y-J., Shlesinger, M., Brown, A., Hamilton, D., Cohen, E., Lavin, P., and McCaffrey, R. 1981. Successful tumour immunotherapy with cimetidine in mice. The Lancet i, 636-638.

Pampiglione, S., Manson-Bahr, P.E.C., Giungi, F., Guinti, G., Parenti, A., and Canestri Trotti, B. 1974. Studies on Mediterranean leishmaniasis. 2. Asymptomatic cases of visceral leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 68, 447-453.

Panush, R.S. 1975. Effects of certain antirheumatic drugs upon normal human peripheral blood lymphocytes. Inhibition of mitogen- and antigen-stimulated incorporation of tritiated thymidine. Arthritis and Rheumatism 18, 418-419.

Pasvol, G., and Wilson, R.J.M. 1982. Malaria and the red cells. British Medical Bulletin 38, 133-140.

Pearson, R.D., Wheeler, D.A., Harrison, L.H., and Kay, H.D. 1983. The immunobiology of leishmaniasis. Review of Infectious Diseases 5, 907-927.

Pearson, R.D., Manian, A.A., Harcus, J.L., Hall, D., and Hewlett, E.L. 1982. Lethal effect of phenothiazine neuroleptics on the pathogenic protozoan Leishmania donovoni. Science 217, 369-371.

Perez, H. 1983. Factors influencing the host response to Leishmania mexicana. In: Cytopathology Of Parasitic Disease. Ciba Foundation Symposium. Pitman Books, London. Pp. 157-173.

Perez, H., Arredondo, B., and Gonzalez, M. 1978. Comparative study of American cutaneous leishmaniasis and diffuse cutaneous leishmaniasis in two strains of inbred mice. Infection and Immunity 22, 301-307.

Perez, H., Labrador, F., and Torrealba, J.W. 1979. Variations in the response of five strains of mice to Leishmania mexicana. International Journal of Parasitology 9, 27-32.

Peters, W., Trotter, E.R., and Robinson, B.L. 1980. The experimental chemotherapy of leishmaniasis, VII. Drug responses of L. major and L. mexicana amazonensis, with an analysis of promising chemical leads to new antileishmanial agents. Annals of Tropical Medicine and Parasitology 74, 321-335.

Perez, H., De La Rosa, M., and Malave, I. 1984. The effect of protein restriction on the development of protective immunity to Leishmania mexicana. Parasite Immunology 6, 285-294.

Phillips, R.S. 1983. Malaria. Edward Arnold Publishers, Ltd. London. 58 pp.

Radelescu, S., Lupascu, G., Ciplea, A.G., and Cernat, M.J. 1971. Existence du flagelle Giardia muris dans les tissus et organes des souris a infestation sponttanee. Archives roumaines de Pathologie Experimentale et de Microbiologie 30, 405-411.

Reed, S.G., Barral-Netto, M., and Inverso, J.A. 1984. Treatment of experimental visceral leishmaniasis with lymphokine encapsulated in liposomes. The Journal of Immunology 132, 3116-3119.

Rees, P.H., Kager, P.A., Ogada, T., and Eeftinck Schattenkerk, J-K.M. 1985. The treatment of kala-azar: A review with comments drawn from experience in Kenya. Tropical and Geographic Medicine 37, 37-46.

Reeves, W.C. 1971. Mosquito vector and vertebrate host interaction. The key to maintenance of certain arboviruses. In: The Ecology And Physiology Of Parasites (Fallis, A.M., Ed.). University of Toronto Press, Toronto. Pp. 223-230.

Ribiero, J.M., Rossignol, P.A., and Spielman, A. 1984. Role of mosquito saliva in blood vessel location. Journal of Experimental Biology 108, 1-7.

Rieckman, K.H. and Silverman, P.H. 1977. Plasmodia of man. In: Parasitic Protozoa, Volume III (Kreier, J.P., Ed.). Academic Press, New York. Pp. 493-528.

Rioux, J.A., Killick-Kendrick, R., Leaney, A.J., Turner, D.P., Lanotte, G. and Bailly, M. 1979. Ecologie des leishmanioses le sud de la France. II. La leishmaniose canine: succes de la transmission experimentale "Chien-Phlebotome-Chien" par la piqure de Phlebotomus ariasi Tonnoir, 1921. Annales de Parasitologie humaine et comparee 54, 401-407.

Rollo, I.M. 1980. Drugs used in the chemotherapy of malaria. In: The Pharmacological Basis Of Therapeutics, 6th Edition (Gilman, A.G., Goodman, L.S., and Gilman, A., Eds.). MacMillan Publishing Co., New York. 1043 pp.

Rossignol, P.A., Ribiero, J.M.C., and Spielman, A. 1984. Increased intradermal probing time in sporozoite-infected mosquitoes. American Journal of Tropical Medicine and Hygiene 33, 17-20.

Rossignol, P.A., Ribiero, J.M.C., Jungery, M., Turell, M.J., Spielman, A., and Bailey, C.L. 1985. Enhanced mosquito blood-finding success on parasitemic hosts: Evidence for vector-parasite mutualism. Proceedings of the National Academy of Science, U.S.A. 82, 7725-7727.

Sacks, D.L. and Perkins, P.V. 1984. Identification of an infective stage of Leishmania promastigotes. Science 223, 1417-1419.

Sacks, D.L. and Perkins, P.V. 1985. Development of infective stage Leishmania promastigotes within phlebotomine sandflies. American Journal of Tropical Medicine and Hygiene 34, 456-459.

Salaman, M.H. 1970. Immunodepression by mammalian viruses and Plasmodia. Proceedings of the Royal Society of Medicine 63, 11-15.

Salaman, M.H., Wedderburn, N., and Bruce-Chwatt, L.J. 1969. The immunodepressive effect of a murine Plasmodium and its interaction with murine oncogenic viruses. Journal of General Microbiology 58, 383-391.

Salmeron, G. and Lipsky, P.E. 1983. Immunosuppressive potential of antimalarials. American Journal of Medicine 75 (Supplement), 1160-1166.

Scherphof, G.L. 1982. Interactions of liposomes with biological fluids and fate of liposomes in vivo. In: Liposome Methodology (Leserman, L.D. and Barbet, J., Eds.). Inserm, Paris. Pp. 80.

Schofield, C.J. 1985. Population dynamics and control of Triatoma infestans. Annales de la Societe Belge de Medecine Tropicale 65 (Supplement 1), 149-164.

Schofield, C.J., and White, G.B. 1982. Density-dependent feeding success in Triatomine bugs and other vectors. In: Ectoparasites Of Veterinary And Medical Importance In Temperate Areas. Royal Army Medical College Symposium. Pp. 1-6.

Schofield, C.J., Williams, N.G., and Marshall, T.F. De C. 1986. Density-dependent perception of Triatomine bug bites. Annals of Tropical Medicine and Parasitology 80, 351-358.

Scott, P. and Sher, A. 1986. A spectrum in the susceptibility of Leishmanial strains to intracellular killing by murine macrophages. The Journal of Immunology 136, 1461-1466.

Seed, T.M., and Kreier, J.P. 1980. Erythrocyte destruction mechanism in malaria. In: Malaria, Volume II (Kreier, J.P., Ed.). Academic Press, New York. Pp. 1-46.

Semprevivo, L.L., DeTolla, L.J., Passmore, H.C., and Palczuk, N.C. 1981. Spectral model of leishmaniasis in congenic strains of mice. Journal of Parasitology 67, 8-14.

Sen Gupta, P.C. 1964. Post kala-azar dermal leishmaniasis. Scientific Reports of the Instituto Sanita 2, 124-130.

Sen Gupta, P.C. and Bhattachargee, B. 1953. Histopathology of post kala-azar dermal leishmaniasis. Journal of Tropical Medicine and Hygiene 56, 110-116.

Seyedi-Rashiti, M.A., and Nadim, A. 1975. Re-establishment of cutaneous leishmaniasis after cessation of anti-malaria spraying. Tropical and Geographical Medicine 27, 79-82.

Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. 593 pp.

Strangways-Dixon, J. and Lainson, R. 1966. The transmission of Leishmania mexicana to man by Phlebotomus pessoanus with observations on the development of the parasite in different species of Phlebotomus. Transactions of the Royal Society of Tropical Medicine and Hygiene 60, 192-207.

Strickland, G.T., Voller, A., Pettit, L.E., and Fleck, D.G. 1972. Immunodepression associated with concomitant Toxoplasma and malarial infections in mice. Journal of Infectious Diseases. 126, 54-60.

Taylor, D.N., Wasi, C. and Bernard, K. 1984. Chloroquine prophylaxis associated with a poor antibody response to human diploid cell rabies vaccine. Lancet i, 1405.

Tempelis, C.H., Hays, R.O., Hess, A.D., and Reeves, W.C. 1970. Blood-feeding habits of four species of mosquitoes found in Hawaii. American Journal of Tropical Medicine and Hygiene 19, 335-341.

Tesh, R.B. and Papaevangelou, G. 1977. Effect of insecticide spraying for malaria control on the incidence of sandfly fever in Athens, Greece. American Journal of Tropical Medicine and Hygiene 26, 163-166.

Thakur, C.P. 1986. Harmful effect of high stibogluconate treatment of kala-azar in India. Transactions of the Royal Society of Tropical Medicine and Hygiene 80, 672-673.

Thakur, C.P., Kumar, M., Singh, S.K., Sharma, D., Prasad, U.S., Singh, R.S., Dhawan, P.S., and Aschari, V. 1984. Comparison of regimens of treatment with stibogluconate in kala-azar. British Medical Journal 288, 895-897.

Thong, Y.H., and Ferrante, A. 1978. Inhibition of mitogen-induced lympocyte proliferative responses by quinine. American Journal of Tropical Medicine and Hygiene 27, 354-356.

Thong, Y.H., Ferrante, A., and Rowan-Kelly, B. 1978. Primaquine inhibits mitogen-induced human lymphocyte proliferative responses. Transactions of the Royal Society of Tropical Medicine and Hygiene 72, 537-539.

Thong, Y.H., Ferrante, A., Rowan-Kelly, B., and O'Keefe, D.E. 1979. Effect of mefloquine on the immune response in mice. Transactions of the Royal Society of Tropical Medicine and Hygiene 73, 388-390.

Thurston, J.P. 1955. Observations on the course of Eperythrozoon coccoides infections in mice, and the sensitivity of the parasite to external agents. Parasitology 45, 141-151.

Titus, R.G., Ceredig, R., Cerrottini, J-C., and Louis, J.A. 1985. Therapeutic effect of anti-L3T4 monoclonal antibody GK1.5 on cutaneous leishmaniasis in genetically-susceptible Balb/C mice. The Journal of Immunology 135, 2108-2114.

Trist, D.G. and Weatherall, M. 1981. Inhibition of lymphocyte transformation by mepacrine and chloroquine. Journal of Pharmacy and Pharmacology 33, 434-438.

Turk, J.L., and Bryceson, A.D.M. 1971. Immunological function in leprosy and related diseases. Advances in Immunology 13, 209-266.

Waage, J.K. and Nondo, J. 1982. Host behavior and mosquito feeding success: An experimental study. Transactions of the Royal Society of Tropical Medicine and Hygiene 76, 119-122.

Walker, E.D. and Edman, J.D. 1985. Feeding-site selection and blood-feeding behavior of Aedes triseriatus (Diptera: Culicidae) on rodent (Sciuridae) hosts. Journal of Medical Entomology 3, 287-294.

Walker, E.D. and Edman, J.D. 1986. Influence of defensive behavior of eastern chipmunks and grey squirrels (Rodentia: Sciuridae) on feeding success of Aedes triseriatus (Diptera: Culicidae). Journal of Medical Entomology 23, 1-10.

Walsh, J.A. and Warren, K.S. 1979. Selective primary health care. An interim strategy for disease control in developing countries. New England Journal of Medicine 301, 967-974.

Walters, L.L., Modi, G.B., Tesh, R.B. and Burrage, T. 1987. Host-parasite relationship of Leishmania mexicana mexicana and Lutzomyia abonnenci (Diptera: Psychodidae). American Journal of Tropical Medicine and Hygiene 36, 294-314.

Walton, B.C., Harper, J., and Neal, R.A. 1983. Effectiveness of Allopurinol against Leishmania braziliensis braziliensis in Aotus trivirgatus. American Journal of Tropical Medicine and Hygiene 32, 46-50.

Walton, B.C., Valverde Chinel, L., and Equia y Equia, O. 1973. Onset of Espundia after many years of occult infection with Leishmania braziliensis. American Journal of Tropical Medicine and Hygiene 22, 696-698.

Warburg, A. and Schlein, Y. 1986. The effect of post bloodmeal nutrition of Phlebotomus papatasi on the transmission of Leishmania major. American Journal of Tropical Medicine and Hygiene 35, 926-930.

Ward, R.D. 1977. New World leishmaniasis: A review of the epidemiological changes in the last three decades. Proceedings of the XV International Congress of Entomology. Pp. 505-522.

Ward, R.D. 1985. Vector biology and control. In: Leishmaniasis (Chang, K.-P., and Bray, R.S., Eds.). Elsevier, New York. Pp. 199-212.

Washino, R.K. and Tempelis, C.H. 1970. Host-feeding patterns of Anopheles freeborni in the Sacramento valley, California. Journal of Medical Entomology 4, 311-314.

Wedderburn, N. 1974. Immunodepression produced by malarial infection in mice. In: Parasites In The Immunized Host: Mechanisms Of Survival. Ciba Foundation Symposium. Elsevier-Excerpta Medica, Amsterdam. Pp. 123-159.

Weinbaum, R.I., Weintraub, J., Knrumah, F.K., Evans, C.B., Tieglaar, R.E., and Rosenberg, Y.J. 1978. Immunity to Plasmodium berghei yoelii in mice. II. Specific and nonspecific cellular and humoral responses during the course of infection. Journal of Immunology 121, 629-636.

Williams, P. 1966. Experimental transmission of Leishmania mexicana by Lutzomyia cruciata. Annals of Tropical Medicine and Parasitology 67, 365-372.

World Health Organization. 1979. World Health Organization Expert Committee On Malaria. World Health Organization Technical Report Series 640. World Health Organization, Geneva. 71 pp.

World Health Organization. 1982. Malarial Control And National Health Goals. World Health Organization Technical Report Series 680. World Health Organization, Geneva. 68 pp.

World Health Organization. 1984a. The Leishmaniases. World Health Organization Technical Report Series 701. World Health Organization, Geneva. 140 pp.

World Health Organization. 1984b. Advances In Malaria Chemotherapy. World Health Organization Technical Report Series 711. World Health Organization, Geneva. 218 pp.

Zapata-Sirvent, R.L., Narrod, J.A., and Hansbrough, J.F. 1985. Restoration of delayed hypersensitivity in mice receiving immunosuppressive drugs by cimetidine. Transplantation 39, 449-450.

Zeldis, J.B., Friedman, L.S., and Isselbacher, K.J. 1983. Ranitidine: A new H_2-receptor antagonist. The New England Journal of Medicine, 309, 1368-1373.

Zuckerman, A. 1977. Current status of the immunology of blood on tissue protozoa. II. Plasmodium. Experimental Parasitology 42, 374-446.

Zuckerman, A. and Lainson, R. 1977. Leishmania. In: Parasitic Protozoa, Volume 1 (Kreier, J.P., Ed.). Academic Press, New York. Pp. 58-134.