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REDUCED EFFECT OF CHEMOTHERAPY OF STRONGYLOIDIASIS IN PATIENTS WITH CONCURRENT HTLV-I INFECTION IN OKINAWA, JAPAN

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Abstract: The effect of concurrent HTLV-I infection on the efficacy of anti-*Strongyloides* chemotherapy has been tested in a prognostic study on 96 patients with uncomplicated strongyloidiasis. The efficacy of treatment with pyrvinium pamoate and thiabendazole was found to be significantly low in the HTLV-I seropositive patients, as compared to that in the seronegative group. The cure rates in the HTLV-I seropositive patients, when assessed by both faecal examination and serological testing, were only 8.6% for pyrvinium pamoate treatment and 35.0% for thiabendazole treatment. On the other hand, the cure rates were 31.3% for pyrvinium pamoate treatment and 57.1% for thiabendazole treatment in the seronegative group.

Although antibody response against *Strongyloides* did not decrease among the HTLV-I seropositive patients, the total serum IgE levels, as well as eosinophil counts in peripheral blood, were relatively low in the seropositive group. Abnormal lymphocytes were demonstrated in 38.5% of the patients with concurrent HTLV-I infection. The presence of abnormal lymphocytes, however, appeared not to correlate with the therapeutic efficacy. A possible connection between the poor efficacy and depressed immune responses provided by the concurrent HTLV-I infection is discussed. The low efficacy in the HTLV-I seropositive patients also provides a causal explanation for the significant accumulation of patients with concurrent HTLV-I infection in Okinawa.

INTRODUCTION

Strongyloidiasis caused by *Strongyloides stercoralis* infection still remains prevalent in Okinawa Prefecture, Japan, where other parasitic diseases have been almost entirely eradicated in recent years (Sato, 1986). Because of its opportunistic nature, the great majority of patients who are immunocompetent are generally chronic, producing no symptom clearly attributable to the presence of the parasite. On the other hand, under the condition of depressed immune competence, the chronic infection progresses to a massive and often fatal systemic infection as a consequence of increasing autoinfection with larval parasites.

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Okinawa Prefecture is also known to be an endemic area for human T-cell leukemia virus (HTLV-I) infection (Clark *et al.*, 1985). The HTLV-I is aetiologically associated with adult T-cell leukemia (ATL) which leads to severe deficiencies in immunological responses. Recently, it has been demonstrated that the patients with an asymptomatic *Strongyloides* infection in Okinawa are highly accompanied by the ATL viral infection (Nakada *et al.*, 1984; Fujita *et al.*, 1985; Sato and Shiroma, 1989). Under the condition of concurrent *Strongyloides* and ATL viral infection, the progression of asymptomatic *Strongyloides* infection to a fatal disseminated state has been often observed among the patients who developed ATL (Takara *et al.*, 1980; Matsui *et al.*, 1982; Oura *et al.*, 1986). A possible explanation for the high frequency of such a complication, however, has not yet been offered. In this paper we present results showing the depressed efficacy of treatment of strongyloidiasis in patients with concurrent HTLV-I infection and thus discuss the possibility that the low therapeutic efficacy may be attributed to the depressed immune responses provided by the viral infection and also may be responsible for a significant accumulation of strongyloidiasis patients with concurrent HTLV-I infection for a long period in Okinawa.

Materials and Methods

Patients

A total of 96 patients with strongyloidiasis received medical treatment for strongyloidiasis in the Izumizaki Hospital, Okinawa, Japan. They were consisted of 47 males and 49 females. Their age ranged from 30 to 74 years (mean=53.3 years). They were asymptomatic or mildly symptomatic cases. Among them, positive rate of antibodies against ATL-associated antigen (ATLA) was as high as 67.7%.

Treatments

Fifty-one patients were treated with pyrvinium pamoate suspension (Poquil), which was administered in a dosage of 5 mg per kg body weight daily for 3 to 5 consecutive days. For the other 27 patients, thiabendazole (25 mg/kg for 4 days) was administered. The above treatment schedules have long been used in Okinawa for treatment of strongyloidiasis. The remaining 18 patients were left without any treatment as a control group. The duration from treatment to follow-up faecal examination was different for each cases, but it was more than 2 years for all cases.

Faecal examination

The follow-up faecal examination after the treatment was performed repeatedly for consecutive 3 days by three different method, e.g. direct faecal smear, formol-ether concentration method and faecal culture (Harada-Mori method).

Antibodies to *S. stercoralis*

In order to assess serologically the efficacy of treatment, the serum antibodies to *S. stercoralis* were measured by an enzyme-linked immunosorbent assay (ELISA). The antigens used were prepared from *S. stercoralis* filariform larvae collected from faeces of strongyloidiasis patients (Sato *et al.*, 1983). The conventional technique for the micro-ELISA using a microtiter plate was the same as that described in a previous paper (Sato *et*

al., 1985). The sera were tested at a single dilution of 1:50 and the intensity of antibody response was expressed as the absorbancy (OD) at 500 nm. On the basis of the previous criteria, the OD value of over 0.5 was regarded as being antibody positive (Sato *et al.*, 1990b).

Detection of antibody to ATL virus

The individuals having anti-ATLA antibodies have been known to be infected with HTLV-I in their peripheral lymphocytes (Gotoh *et al.*, 1982).

A kit for the particle agglutination test to detect anti-ATLA antibodies was kindly supplied from Fujirebio Inc., Tokyo. A usual indirect agglutination test using gelatin particles coated with antigens prepared from culture fluid of the virus-producing cell line was done in U-bottomed wells of a plastic microplate (Ikeda *et al.*, 1984). The mixtures of the antigen-coated particles and serially diluted sera in the wells were allowed to stand for 3 hr at room temperature and the resulting patterns formed on the bottom of the wells were read. The final serum dilution of 1:16 or higher showing agglutination was interpreted as positive.

Serum total IgE

Serum IgE was measured by a standard radioimmunosorbent test using Phadebas IgE test kit (PRIST; Pharmacia Fine Chemicals, Uppsala, Sweden). Serum IgE levels were expressed in ng/m*l* of serum, assuming that 1 unit/m*l* corresponds to approximately 2 ng/m*l*.

Haematological examinations

Eosinophils from peripheral blood were counted in Wright's-stained smear and were reported as percentage in 100 leukocytes. Morphologically abnormal lymphocytes having lobular or indented nulei were also counted in the same smears.

Statistics

The data were analyzed by χ^2 test and Student's t test, as appropriate. A P value of more than 0.05 was considered not to be significant.

RESULTS

The results of follow-up examination after treatment are shown in Table 1. The total cure rates, as estimated by both faecal and serological examinations, were 15.7% for pyrvinium pamoate treatment and 40.7% for thiabendazole treatment, respectively. Although three patients in the untreated group were negative in the follow-up faecal examination, they were serologically equivocal for complete cure and the spontaneous cure determined by exclusion of the equivocal cases was not detected in the untreated group. When the efficacy were compared between the HTLV-I seropositive and seronegative patients, the cases with complete cure were significantly fewer in the HTLV-I seropositive group than in the seronegative group in both treatments.

Table 2 represents total IgE levels in sera of 39 patients who were still positive after the treatment. The IgE levels were relatively lower in the HTLV-I seropositive group than in the seronegative group. Although the difference was not significant statistically, the mean IgE level in the seropositive group was as low as one-third that of the seronegative group.

Table 1 Effect of concurrent HTLV-I infection on treatment of strongyloidiasis with pyrvinium pamoate and thiabendazole

Treatment	Anti-ATLA antibody	No. examined	Faecal examination: Positive (not cured)	Faecal examination: Negative — Sero-positive† (equivocal)	Faecal examination: Negative — Sero-negative† (cured)	Significance*
Pyrvinium pamoate	Positive	35	30 (85.7)	2 (5.7)	3 (8.6)	P<0.05
	Negative	16	10 (62.5)	1 (6.3)	5 (31.3)	
	Total	51	40 (78.4)	3 (5.9)	8 (15.7)	
Thiabendazole	Positive	20	9 (45.0)	4 (20.0)	7 (35.0)	N.S.
	Negative	7	2 (28.6)	1 (14.3)	4 (57.1)	
	Total	27	11 (40.7)	5 (18.5)	11 (40.7)	
Untreated	Positive	10	7 (70.0)	3 (30.0)	0 (0)	N.S.
	Negative	8	8 (100)	0 (0)	0 (0)	
	Total	18	15 (83.3)	3 (16.7)	0 (0)	

*Significance between HTLV-I seropositive and seronegative groups; †anti-*Strongyloides* antibody

Although the difference of cure rate between HTLV-I seropositive and seronegative groups was not significant ($P<0.1$) in thiabendazole treatment, the total cure rate of both treatments was significantly lower ($P<0.05$) in the HTLV-I seropositive group.

Table 2 Comparison of serum total IgE levels between HTLV-I seropositive and seronegative patients

IgE level (ng/m*l*)	Anti-ATLA antibody: Positive (n=38)	Anti-ATLA antibody: Negative (n=12)	
<1,000	34 (89.5)	8 (66.7)	
1,000 - 3,000	4 (10.5)	2 (16.7)	
3,100 - 5,000	0 (0)	1 (8.3)	
>5,000	0 (0)	1 (8.3)	
Mean±SD	415.8±523.2	1,300±1,795	N.S.*

*Significance between HTLV-I seropositive and seronegative groups.

Table 3 also shows eosinophil counts in peripheral blood of 25 patients with *Strongyloides* infection. Eosinophilia of over 6% was detected in 57.8% of patients who were negative for anti-ATLA antibody, while the percentage of patients with eosinophilia was 35.0% in the HTLV-I seropositive patients. On the other hand, when anti-*Strongyloides* antibodies were compared between the HTLV-I seropositive and seronegative groups, the antibody levels were relatively higher in the seropositive group, showing no depressed antibody response to *Strongyloides* among the seropositive group (Fig. 1).

The presence of abnormal lymphocytes in peripheral blood was determined in 52 patients who were positive for anti-ATLA antibody and atypical lymphocytes were detected in 20

Table 3 Eosinophil counts in peripheral blood of strongyloidiasis patients with or without concurrent HTLV-I infection

Blood eosinophils (%)	Anti-ATLA antibody		
	Positive (n=40)	Negative (n=19)	
-6	26 (65.0)	8 (42.1)	
7-10	7 (17.5)	7 (36.8)	
11-15	6 (15.0)	2 (10.5)	
16-	1 (2.5)	2 (10.5)	
Eosinophilia (>6%)	14 (35.0)	11 (57.8)	N.S.*

*Significance between HTLV-I seropositive and seronegative groups.

(38.5%) patients. The relation between the presence of atypical cells and the therapeutic efficacy is shown in Table 4. The positive rate of atypical cells was relatively higher in the group of unsuccessful treatment than in the group of complete cure in pyrvinium pamoate treatment, but it were almost similar in thiabendazole treatment.

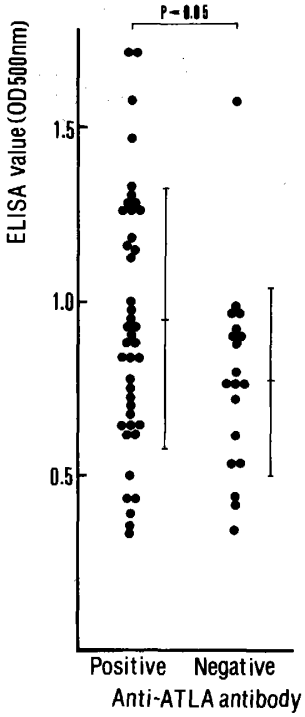

Figure 1 Comparison of anti-*Strongyloides* ELISA values between HTLV-I seropositive and seronegative patients with strongyloidiasis.

Discussion

Strongyloidiasis and ATL are presently highly prevalent in Okinawa Prefecture, Japan; the prevalence levels appear to be 5 to 10% for *Strongyloides* (Sato, 1986; Sato *et al.*, 1990b) and about 20% for HTLV-I infection (Clark *et al.*, 1985). Recently, it has been pointed out by several researchers that *Strongyloides* carriers in Okinawa are frequently accompanied by HTLV-I infection (Nakada *et al.*, 1984; Fujita *et al.*, 1985; Sato and Shiroma, 1989). In these studies, the positive rates of anti-ATLA antibody as high as

Table 4 Correlation between efficacy of treatment and presence of abnormal lymphocytes in peripheral blood of patients with concurrent HTLV-I infection

Treatment	Efficacy of treatment			Total
	Not cured	Equivocal	Cured	
Pyrvinium pamoate	13/28 (46.4)	0/2 (0)	1/3 (33.3)	14/33 (42.4)
Thiabendazole	3/9 (33.3)	1/3 (33.3)	2/7 (28.6)	6/19 (31.6)
Total	16/37 (43.2)	1/5 (20.0)	3/10 (33.3)	20/52 (38.5)

No. positive/No. examined (%)

57.8 to 73.6% were detected among the strongyloidiasis patients.

Several explanations have been proposed for the severe complication of these infections. The participation of antigenic components common to *Strongyloides* and ATLA which may produce high positive rate of anti-ATLA antibody among the *Strongyloides* carriers has been excluded by the authors (Sato and Shiroma, 1989). Possible participation of any epidemiological factors to produce the severe overlap of the two infections has also been excluded in a previous paper (Sato *et al.*, 1990a).

Alternatively, it can be supposed that the concurrent HTLV-I infection may affect on the severity of *Strongyloides* infection through the depressed immune competence of host during the viral infection. The data presented in this paper demonstrate that the anti-parasitic effect of the anthelmintics is greatly reduced in strongyloidiasis patients with concurrent HTLV-I infection. In Okinawa, intractable cases of strongyloidiasis, in which relapse occurs repeatedly after various treatment over a period of many years, have been often observed (Shiroma *et al.*, 1990). Although the factor responsible for such a resistance to anthelmintic treatment is unclear, it can be postulated that the poor efficacy of treatment may be attributed to the depressed immune response which provided by the viral infection. As well documented, ATL is characterized by an unique T-lymphocyte malignancy which leads to severe deficiencies in immune responses, and it is also known that the disorder to a T-cell mediated immune system has already begun in the stage of virus carriers (Imai and Hinuma, 1983; Yasuda *et al.*, 1986; Tanaka *et al.*, 1989; Prince *et al.*, 1990). Because of the opportunistic nature of the parasitic pathogen, it is reasonable to suppose the immune dependence of anti-parasitic chemotherapy in strongyloidiasis.

In the past decade, substantial evidence for the immune dependence of chemotherapy has accumulated. It has been known that immunosuppression reduces the efficacy of chemotherapy in several parasitic diseases, such as malaria (Lwin *et al.*, 1978), trypanosomiasis (Frommel, 1988), onchocerciasis (Bianco *et al.*, 1986) and schistosomiasis (Doenhoff and Bain, 1978). In the case of strongyloidiasis, it is also well documented that the patients with severe infection under the immunocompromised condition often fail to respond to anthelmintic treatment, and that repeated courses of treatment are necessary to obtain a complete cure for such a severe case (Scowden *et al.*, 1978; Weller *et al.*, 1981; Shelhamer *et al.*, 1982; Morgan *et al.*, 1986). Although the immune factor involved in influencing the drug efficacy is not yet determined, it has been reported that antibodies specific for parasite, when it were administered simultaneously with drug, enhanced the efficacy of drug against schistosomes and malaria parasites (Doenhoff and Bain, 1978; Targett, 1985; Brindly and Sher, 1987). When the antibody titers to *Strongyloides* were compared between HTLV-I seropositive and seronegative patients in the present study, however, we could not find any evidence to suppose a depressed antibody response against *Strongyloides* in the seropositive group. While the drug efficacy in onchocerciasis reduced markedly in T-cell deprived mice but not in congenitally B-cell deficient mice, suggesting that cellular immune effector mechanism other than the serum antibody may contribute to the efficacy of chemotherapy in this parasitic disease (Bianco *et al.*, 1986). With respect to strongyloidiasis, significance of cell-mediated immunity in controlling and preventing *Strongyloides* infection has also been suggested in several clinicopathological studies (Purtilo *et al.*, 1974; Scowden *et al.*, 1978; Cohen and Spry, 1979). As already mentioned above, HTLV-I carriers are known to have immunological abnormalities, such as increased spontaneous lymphoproliferation, decreased mitogenic responses and

increased expression of IL-2 receptor on the surface of T lymphocytes. In the present study, morphologically atypical lymphocytes were detected in about 40% of HTLV-I seropositive patients examined. In their study on 36 patients, Nakada *et al.* (1987) has reported that abnormal lymphocytes, as well as monoclonal integration of HTLV-I proviral DNA, were detected in many patients and indicated that the presence of abnormal cells and monoclonal integration of proviral DNA correlated with a trend for greater severity of the parasitic infection. However, we could find no correlation between the presence of abnormal cells and the efficacy of treatment in the present study. On the other hand, it was noted that total serum IgE levels and eosinophil counts in peripheral blood were relatively low in the HTLV-I seropositive patients. The IgE and eosinophilic responses are well known to play an important role in protective immunity to parasitic helminths (Capron *et al.*, 1981; Dessein *et al.*, 1981; Kojima *et al.*, 1985). Recently, it was also demonstrated in a study with murine infection model that IL-5-dependent eosinophilic response was important for protective immunity to *Strongyloides* infection (Korenaga *et al.*, 1991). The relative decrease of IgE level and peripheral eosinophils in the HTLV-I seropositive patients may participate in the poor efficacy of chemotherapy. Further investigations on a possible connection between the immune responses and drug efficacy in strongyloidiasis should be intended.

Finally, the reduced efficacy of drug treatments in HTLV-I seropositive patients may provide another explanation for the high concurrency of HTLV-I. Due to resistance to anthelmintic treatments, which has long been used for strongyloidiasis in Okinawa, the patients with concurrent HTLV-I infection might harbour the infection for many years, resulting a significant accumulation of such patients.

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糞線虫症の治療効果に及ぼすATLウイルス混合感染の影響

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沖縄では糞線虫保有者の多くに，ATL病原ウイルスの混合感染が見られることが知られている。ATLウイルスの混合感染が，糞線虫に対する駆虫効果にどのような影響を与えているかについて，96名の糞線虫保有者を対象に検討した。治療はピルビニーム・パモエート（PP）とサイアベンダゾール（TB）を用いて行い，いずれの場合でもATLウイルスの感染が陽性である糞線虫保有者において，治療後の治癒率が著明に低いことを確認した。すなわち，ATLウイルス陽性者での治癒率は，PP治療でわずか8.6%，TB治療の場合で35.0%であったのに対し，ATLウイルス陰性の糞線虫保有者では，おのおの31.3%，57.1%であった。

これらの糞線虫保有者について，抗-糞線虫抗体値を比較したところ，ATLウイルス陽性群において抗体値が低いことを確認することはできなかったが，末梢血好酸球と血清総IgEレベルは，ATLウイルス陽性群において低い傾向が認められた。また，ATLウイルス陽性の対象者において，その約40%に異型リンパ球の出現を認めたが，異型リンパ球の存在と駆虫効果の間には特に関連を認め得なかった。かかる治癒率の低下は，ATLウイルスの感染による何らかの免疫低下状態によってもたらされた可能性と，これが長年にわたりATLウイルス陽性の糞線虫保有者を選択的に蓄積させ，今日の高い混合感染状態をもたらした，主要な原因である可能性が考えられた。

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ANALYSIS OF DRINKING WATER IN AIRPORTS, AIRPLANES AND PUBLIC WATER FACILITIES

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Abstract: The results indicated that drinking water samples from 4 (44.4%) of 9 airports and from 2 (22.2%) of 9 airplanes gave a positive qualitative test for at least any one of ammoniacal nitrogen, nitrite nitrogen and nitrate nitrogen. Samples from 8 (57.1%) of 14 airports, from 8 (66.7%) of 12 airplanes and from 10 (62.5%) of 16 public facilities showed a value for the consumption of potassium permanganate of 20 mg/*l* or above. Those from 5 (31.3%) of 16 airports, from 3 (18.8%) of 16 airplanes and from 3 (15.0%) of 20 public facilities had a hardness of 200 mg/*l* or above. A total bacterial count of 1.0×10^2/m*l* or above was obtained for samples from 6 (66.7%) of 9 airports, from 7 (63.6%) of 11 airplanes and from 11 (61.1%) of 18 public facilities; a positive coliform group culture was noted for samples from 5 (62.5%) of 8 airports, from 6 (60.0%) of 10 airplanes and from 4 (36.4%) of 11 public facilities. Residual chloride was not detected in any measurable amount in samples from 9 (56.3%) of 16 airports, from 2 (40.0%) of 5 airplanes and from 4 (57.1%) of 7 public facilities.

Bacteriological examination of 29 samples positive for total bacteria and coliform bacilli revealed that *Escherichia coli* was detected most frequently, followed by *Acinetobacter calcoaceticus, Aeromonas hydrophilia, Pseudomonas* spp., *Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae* and *Citrobacter freundii* in that descending order.

Striking correlation were noted to exist between negativity for residual chloride and positivities for total bacteria or coliform group; and then positivities for ammoniacal nitrogen, nitrite nitrogen, nitrate nitrogen, a heightend value of the consumption of potassium permanganate and positivities for total bacteria or coliform group.

INTRODUCTION

The number of Japanese traveling abroad has reportedly been increasing steadily to exceed 10 million per year in these latest years. As a natural consequence, persons who are affected with a diarrheal or feverish disease while staying abroad or after returning home are also on the increase and this growing population with so-called imported infections poses a

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serious socio-medical ploblem (Okumura and Nunode, 1980; Kimura *et al.*, 1987; Weinke *et al.*, 1988). Of these infectious diseases, the main source of those involving the digestive organs, needless to say, is insanitary foods and drinks, plays a major etiologic role (Sunaga and Sawada, 1986; Okumura *et al.*, 1990).

From this viewpoint, we have carried out analysis of drinking water offered to Japanese residents as well as natives in under-developed countries and reported the results thus obtained during the past decade (Okumura and Watanabe, 1985; Okumura, 1986). In the present study, we undertook analyses, with bacteriological examination, of those types of drinking water to which travelers in general can easily gain access, i.e. offered in airplanes, in airports and via public water facilities in noted places for sight-seeing in an effort to contribute to the prevention of water-borne infections occurring during overseas traveling. The purpose of this communication is to present the finding thus yield.

Materials and Methods

Chosen by us for examination were samples of drinking water serviced in airplanes, from water coolers at the airports and from water faucets at railway stations, parks, noted places and historic sites. These samples were analyzed or determined for ammoniacal nitrogen, nitrite nitrogen, nitrate nitrogen, chloric ion, residual chloride, consumption of potassium permanganate, fluorine and total hardness in accordance with "Method of Water Analysis" - Explanation of Hygienic Test Methods ed. the Pharmaceutical Society of Japan.

For quantification of total bacteria and coliform group, water specimens were filtered through membrane filters (Millipore, Water-tester MT 25 & MC 25, Millipore Corp.), washing of the filters were incubated in media for 24-48 hr at 37°C and colonies formed were counted. Specimens were also incubated in blood agar, BTB and Butzler's medium for the formation of colonies, which submitted themselves to identification procedures utilizing media for identification (TSI-agar, etc.) and simple identification kits (Thermo Co., Ltd., BL-E21 Enterogram, BL-N18 Nonfagram, etc.) (Pharmaceutical Society of Japan, 1983).

Results

Drinking water samples from water coolers installed in 4 (44.4%) of 9 airports (including their boarding lounges), from 2 (22.2%) of 9 airplanes and from 10 (56.2%) of 16 public water facilities (railway station and noted place) studied were found positive, both qualitatively and quantitatively, for all or any one or two of ammoniacal nitrogen, nitrite nitrogen and nitrate nitrogen.

High values for the consumption of potassium permanganate in excess of 20 mg/*l* were obtained for drinking water samples from 8 (57.1%) of 14 airports, from 8 (66.7%) of 12 airplanes and from 10 (62.5%) of 16 public water facilities studied.

A total hardness of 200 mg/*l* or greater was noted for drinking water samples from 5 (31.3%) of 16 airports, from 3 (18.8%) of 16 airplanes and from 3 (15.0%) of 20 public water facilities.

Drinking water samples from 6 (66.7%) of 9 airports, from 7 (63.6%) of 11 airplanes and 11 (61.1%) of 18 public water facilities were shown to contain 1.0×10^2/m*l* of total bacteria. Drinking water samples from 5 (62.5%) of 8 airports, from 6 (60.0%) of 10 airplanes and from

Table 1 Ratio of abnormality on chemical and biological test in drinking water

	Airport (%)	Airplane (%)	Water facilities (%)	Total (%)
NH_3-N, NO_2-N, NO_3: positive	4/9 (44.4)	2/9 (22.2)	9/16 (56.2)	15/34 (44.1)
$KMnO_4$: over 20 mg/*l*	8/14 (57.1)	8/12 (66.7)	10/16 (62.5)	26/42 (61.9)
Hardness: over 200 mg/*l*	5/16 (31.3)	3/16 (18.8)	3/20 (15.0)	11/52 (21.2)
Total bacteria: positive	6/9 (66.7)	7/11 (63.6)	11/18 (61.1)	24/38 (63.2)
Coliform group: positive	5/8 (62.5)	6/10 (60.0)	4/11 (36.4)	15/29 (51.7)
Residual Cl: negative	9/16 (56.3)	2/5 (40.0)	4/7 (57.1)	15/28 (53.6)

4 (36.4%) of 11 public water facilities proved positive for coliform group.

Also samples from 9 (56.3%) of 16 airports, from 2 (40.0%) of 5 airplanes and 4 (57.1%) of 7 public water facilities were noted to be negative for residual chloride on qualitative testing (Table 1).

Bacteriological examination of colonies of total bacteria and coliform group with culture media for their identification and simple identification kits revealed that *E. coli* was detected in drinking water samples from 11 sources (5 airports, 4 airplanes and 2 public water facilities), *A. calcoaceticus* from 6 sources, *A. hydrophila* from 4 sources, *Pseudomonas* spp. from 4 sources, *K. pneumoniae* from 3 sources, *P. mirabilis* from 2 sources, *E. cloacae* from 2 sources, *C. freundii* from 2 sources, and non-haemolytic streptococci, *Bacillus* spp., *Staphylococcus epidermidis,* NF-GNR (dextrose non fermentant Gram-negative rods), *Campylobacter jejuni, Chromobacterium* spp., *Klebsiella oxytoca, Hafnia alvei* and *Acinetobacter lwoffii* from 1 source each (Tables 2, 3, 4 and 5).

Table 2 Bacterial dose and isolated stains from drinking water in airports

Airport	Dose (CFU/m*l*)	Isolated strains
A	3.0×10^6	*Acinetobacter calcoaceticus, Escherichia coli*
B	1.6×10^6	*A. calcoaceticus,* non-haemolytic streptococcii
C	2.7×10^6	*Aeromonas hydrophila, E. coli, Bacillus* sp.
D	2.0×10^5	*E. coli*
E	8.2×10^5	*Pseudomonas* sp., *Proteus mirabilis*
F	7.4×10^3	*Pseudomonas cepacia*
G	1.0×10^4	*E. coli, Pseudomonas* sp.
H	2.8×10^4	*E. coli*
I	3.0×10^3	*Enterobacter cloacae*

(media : MacConkey-agar, YCA, DHL, BTB)

Table 3 Bacterial dose and isolated strains from drinking water in airplanes

Airplane	Dose (CFU/ml)	Isolated strains
A	7.0×10^{2}	*E. coli, Staphylococcus epidermidis, Citrobacter freundii*
B	1.0×10^{2}	*E. coli*
C	5.0×10^{6}	*A. calcoaceticus, A. hydrophila*
D	5.0×10^{7}	*Pseudomonas* sp., *Klebsiella pneumoniae*
E	3.0×10^{2}	*E. coli, E. cloacae*
F	7.0×10^{2}	*E. coli*
G	4.3×10^{5}	*Campylobacter jejuni, Chromobacterium* sp.
H	7.8×10^{5}	*P. mirabilis,* NF-GNR*

(media : MacConkey-agar, YCA, DHL, BTB)
*: dextrose non fermentant Gram-negative rods

Table 4 Bacterial dose and isolated strains from drinking water in public water facilities

Place	Dose (CFU/ml)	Isolated strains
Bus stop in Thailand	3.2×10^{3}	*K. pneumoniae*
National park in Moritania	2.0×10^{3}	*Acinetobactor lwoffii, Hafnia alvei*
Oasis in Argeria	2.0×10^{4}	*E. coli*
National park in Ethiopia	1.5×10^{3}	*A. hydrophila*
Water job in Nigeria	2.0×10^{6}	*A. calcoaceticus, C. freundii*
Central park in Tanzania	2.0×10^{3}	*Klebsiella oxytoca*
Central park in Iraq	6.4×10^{4}	*A. calcoaceticus, E. coli*
Market in Saudi Arabia	2.0×10^{3}	*A. calcoaceticus*
Central park in Israel	5.0×10^{6}	*A. hydrophila*
Park in Pakistan	1.4×10^{3}	*K. pneumoniae*

(media : MacConkey-agar, YCA, DHL, BTB)

The relationships between positivities for total bacteria and coliform group vs. nitrogenous compounds, consumption of potassium permanganate and residual chloride are shown in Table 6, indicating that the ratio of drinking water positive to negative for ammoniacal nitrogen, nitrite nitrogen and nitrate nitrogen was 9:5 for samples positive for total bacteria and 6:2 for those positive for coliform group. Similarly, the ratio of drinking water samples showing a consumption of potassium permanganate of $\leqq 20$ mg/l to those with a corresponding value of >20 mg/l was 10:6 for samples positive for total bacteria and 7:3 for those positive for coliform group. The positive-to-negative ratio of samples for residual chloride was 9:1 and 8:0, respectively, for those positive for total bacteria and coliform group (Table 6).

Table 5 Total of isolated strains in each place

	Airport	Airplane	Public water facilities	Total
E. coli	5	4	2	11
A. calcoaceticus	2	1	3	6
A. hydrophila	1	1	2	4
Pseudomonas sp.	3	1	0	4
K. pneumoniae	0	1	2	3
P. mirabilis	1	1	0	2
E. cloacae	1	1	0	2
C. freundii	0	1	1	2
non-haemolytic streptococcii	1	0	0	1
Bacillus sp.	1	0	0	1
S. epidermidis	0	1	0	1
NF-GNR	0	1	0	1
C. jejuni	0	1	0	1
Chromobacterium sp.	0	1	0	1
K. oxytoca	0	0	1	1
H. alvei	0	0	1	1
A. lwoffii	0	0	1	1

Table 6 Correlation between chemical test and isolated bacteria

		Airport		Airplane		Public water facilities		Total	
		Total Bacteria	Coliform group	Total Bacteria	Coliform group	Total Bacteria	Coliform group	Total Bacteria	Coliform group
NH_3-N, NO_2-N, NO_3-N	positive	3	2	1	2	5	2	9	6
	negative	1	1	3	1	1	0	5	2
$KMnO_4$ 20 mg/*l*:	over	3	3	2	3	5	1	10	7
	under	3	2	1	1	2	0	6	3
Residual Cl:	negative	6	5	1	2	2	1	9	8
	positive	0	0	0	0	1	0	1	0

Discussion

While the main source of intestinal infection in Japanese travelers and residents in foreign countries is contaminated foods and drinks in many instance, it is an undeniable fact that contaminated drinking water also plays a causative role not infrequently. From this viewpoint we have carried out analyses of drinking water mainly in underdeveloped countries during the past 10 years and, based on the results thus obtained, have referred to possible relations between regional peculiarities and the quality of drinking water in climatic zones of vegetation (Okumura, 1986).

In the present study which was conducted over a 10 year period from 1981 to 1991,

samples of drinking water readily accessible to Japanese tourists while traveling abroad, e.g., offered in airplanes, airports and sight-seeing place of various countries, were collected, analyzed and examined bacteriologically in an attempt to contribute to the prevention of drinking water-bone bacterial infections occurring in Japanese travelers in foreign countries.

Testing for the quality of drinking water is done in two ways, i.e. by an environmental research to investigate water flow, geographic and geologic features and the way of using and treating water and by physicochemical tests of water, and on the basis of the results of these studies combined the potability of water and the most appropriate methods of treating and using it are determined. Therefore, with the intention of supplying water of good quality, Article 4 of Japanese Water Works Law (Health & Welfare Ministry's Ordinance No. 56, August 1978) stipulates quality standards of drinking water. While official quality standards of drinking water very somewhat from one country to another, the Japan's law requires that the content of nitrite nitrogen and of nitrate nitrogen be below 10 mg/*l*, although there is no regulation content of ammoniacal nitrogen. The consumption of potassium permanganate must be below 10 mg/*l*, free residual chloride must be 0.1 mg/*l* or above and the hardness be less than 300 mg/*l*. The absence of coliform bacteria and less than 100 of total bacteria per mililiter are mandatory.

However, ammoniacal nitrogen which results from contamination with excreta, factory drainage, etc. is a valuable index for estimation of water contamination. In addition, nitrite nitrogen which is generated mainly by oxidation of ammoniacal nitrogen produced by contamination with excreta and sewage and nitrate nitrogen which is an end-product of oxydation of various nitrogenous compounds are also hallmarks of hygienic importance. The fact that drinking water samples from 15 (44.1%) of 34 facilities studied were positive for all three or any one or two of these forms of nitrogen (even though a substantial number of samples gave less than the regulation value) may be considered as suggesting the possibility that samples were contaminated to some extent.

The Germany's standards require that the consumption of potassium permanganate be below 20 mg/*l*. Drinking water samples from 26 (61.9%) of 42 facilities showed higher values even compared to this rather conservative standard level. The consumption of potassium permanganate usually is increased by contamination with sewage, factory drainage or urine and feces, though occasionally by reasons of geological factors.

The presence of residual chloride in demonstrable quantities is an indication of the efficiency with which drinking water has been sterilized. The fact that samples from 15 (53.6%) of 28 facilities were negative for residual chloride can be constructed as evidencing that chlorination of drinking water as an administrative procedure was not practiced at all or only incompletely.

Moreover, a more than 1.0×10^2 cell count was noted of total bacteria for drinking water samples from 24 (63.2%) of 38 facilities and coliform group for those from 15 (51.7%) of 29 facilities. This fact implies that tap water offered at these facilities was inadequately sterilized or was contaminated with excreta of man and/or animal or some other agents or organisms in the water distribution system. A comparative study of positivities for total bacteria and coliform group vs. nitrogenous compounds, consumption of potassium permanganate and residual chloride showed that drinking water samples that were found positive for nitrogenous compounds as well as those showing higher values for the consumption of potassium permanganate gave higher positive rates for total bacteria and coliform group

bacilli. As a matter of course, drinking water samples without detectable free residual chloride were found to contain a larger number of total bacteria and coliform bacteria. A total bacteria count of 2.0×10^3/m*l* was obtained for a drinking water sample from a public water facility that was positive for residual chloride and this was considered as due presumbly to contamination occurring in the water distribution or delivery system.

Bacterial species detected in the present study were as listed in Table 5. As can be seen, *E. coli* was most frequently, probably as a result of contamination with urine and feces from unknown causes. In addition, *A. calcoaceticus, A. hydrophila, Pseudomonas* spp., *K. pneumoniae, P. mirabilis, E. cloacae* and *C. freundii* were also detected. All these bacterial species were found to bear a close resemblance to strains detected from drinking water in Indonesia by Okuwaki *et al.* (Okuwaki *et al.*, 1982, 1985).

Bacterial infection depends for its establishment upon the virulence of the infecting strain, the pathogenicity of the infecting bacterial species and quantity of the infecting organisms. In other words, its occurrence is determined by the onset dose-to-host response relationship, hence in accordance with dose-response curve. Therefore, when infecting organisms are weak in virulence and minimal in amount and when there are enough host resistance and acquired immunity, a clinically manifest infection, e.g., of intestinal tract, can never be caused. However, it would seem important to recognize drinking water from which infecting agents have been detected to serve at least as a source or route of contamination even if it does not constiture a source or route of infection. In fact, according to Fujita *et al.*, there was a atriking correlation between long-term intact of coliform bacteria-positive drinking water and the positive rate of intestinal parasitosis (Fujita *et al.*, 1982). Traveler's affection with diarrheal disease spoken of so often can be constructed as due to biological factors (i.e. the amount and virulence of infecting agents introduced and decreased immune response of host mentioned above), biochemical changes *in vivo* caused by intake of drinking water of high chlorine concentration and/or of high Mg^{++} concentration (high hardness) and psycho-psychological factors (inadaptability to environmental changes and alien culture).

Thus, in order to get safe and good drinking water, it is most recommendable to boil water, which is the most reliable and easy-to-use method of obtaining potable water. Boiling time is 5 min at level land, increasing by 1 min for each 1,000 m increment of altitude. Boiling of water for 30 min at 60°C will usually be destructive to ordinary pathogenic organisms. If no fuel is available for boiling water, use of water filter (activated charcoal covered with silver carbon) or sodium hypochlorite (the simplest method of purifying drinking water) is highly advisable as a convenient alternative. To add one drop of commercialized sodium hypochlorite solution to approximately 180 m*l* of drinking water and just wait for 5 min will suffice for making it potable. Instead, addition of 4–5 drops of 2% iodine tincture to one liter of drinking water and subsequent allowing it to stand for about 15 min will also serve the same purpose. The resulting aqueous solution will surely be destructive to parasitic ova and cysts, if present at all, without doing any harm to the body (Okumura, 1989).

In summary, our present study revealed that drinking water offered in airplanes and via public water facilities surprisingly was not sanitary enough but contaminated to some varying degrees. It is earnestly hoped that health authorities concerned will make all-out efforts to supply the public with safe drinkable water. Man craves for water by instinct when thirsty. Even though healthy persons are confident of their resistance to casual infection of intestinal tract, it is of utmost importance from the standpoints of preventive medicine and

personal hygienics to know that drinking water offered by readily accessible public facilities is likely to be contaminated with pathogenic organisms. Such awareness will certainly prove of great aid in making a safe and pleasant overseas tour as well as in living a sound and meaningful life in foreign countries.

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航空輸送機関および外国各地公共施設における飲料水の水質

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1981年より1991年の10年間，一般旅行者がごく手軽に飲料水の提供をうけ易い航空機内や空港内，また観光地など公共水飲み施設の飲料水について水質検査を実施し，特に細菌学的検査成績を通して海外旅行中における飲料水による感染病防止について考察した。

アンモニア性窒素，亜硝酸性および硝酸性窒素のいずれかが定性試験で陽性を示したものは，空港では9空港中4空港で44.4%，航空機は9機中2機で22.2%，公共施設は16施設中9施設で56.2%であった。過マンガン酸カリ消費量20 mg/*l* 以上を示したものは，空港では14空港中8空港57.1%，航空機は12機中8機66.7%，公共施設は16施設中10施設62.5%であった。

一般細菌が1.0×10^2/m*l* 以上の集落を認めたものは，空港では9空港中6空港66.7%，航空機は11機中7機63.6%，公共施設は18施設中11施設61.1%であった。大腸菌群が陽性を示したものは，空港では8空港中5空港62.5%，航空機は10機中6機60.0%，公共施設は11施設中4施設36.4%であった。残留塩素の存在が証明し得なかったものは，空港では16空港中9空港56.3%，航空機は5機中2機40.0%，公共施設は7施設中4施設57.1%であった。

一般細菌および大腸菌群陽性の29検体について細菌学的検査を行ったところ，*Escherichia coli* が最も多く検出され，次いで *Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Pseudomonas* spp., *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, *Citrobacter freundii* の順であった。

一般細菌や大腸菌群が陽性を示した飲料水と，アンモニア性窒素，亜硝酸性および硝酸性窒素が陽性を示した飲料水，過マンガン酸カリ消費量が高値の飲料水，そして残留塩素が陰性を示した飲料水との相関は高かった。

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HISTOPATHOLOGICAL OBSERVATIONS OF GOLDEN HAMSTERS INFECTED WITH AN ECUADORIAN ISOLATE OF *LEISHMANIA MEXICANA*

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Abstract: An experimental study was performed to investigate the *Leishmania mexicana* infection in golden hamsters. The animals were infected with *L. mexicana* from Ecuador. At the autopsy 6 months after inoculation, the inoculated sites were shallow, ulcerative and covered with thick crusts. No cutaneous metastasis was observed on other exposed parts of the body. Histologically, specimens of both the nose and footpads showed large numbers of amastigotes with extensive infiltration of histiocytes and lymphocytes and, to some extent, of neutrophils, eosinophils and plasma cells. Large numbers of mast cells were evident in the upper and lower dermis of granulomatous lesions. Amastigotes were found in the macrophages inside the large parasitophorous vacuoles, mostly at the central part of the lesion. Amastigotes were also observed in the liver and spleen by electron microscope but the number was fewer in visceral than in cutaneous sections. Regular destruction of parasites was observed within macrophages in all the cutaneous and visceral sections indicating the phagocytizing role of these cells against parasites.

INTRODUCTION

American cutaneous leishmaniases, caused by *Leishmania mexicana* and *L.* (*Leishmania*) *braziliensis* complex, are widely endemic in Central and South America. Among the many species of the New World *Leishmania, L. mexicana* (=*L. mexicana mexicana*) is well known to be responsible for a variety of disease forms that show everything from localized simple mild lesions to generalized diffuse lesions and that are distributed widely from lowlands to Andean highlands. In Ecuador, the species *L. mexicana* was isolated from both Andean highland and Pacific lowland patients (Armijos *et al.*, 1990; Hashiguchi *et al.*, 1991). However, the clinical manifestations caused by *L. mexicana* had a tendency to vary depending on several unknown factor(s), such as the geographical distribution and immunological

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or physiological conditions of human hosts (Hashiguchi *et al.*, 1991 our unpublished data). In order to investigate the factor(s) involved, we launched a comparative study of Ecuadorian leishmaniasis between the lowlands and highlands, carrying out histopathological and electromicroscopical observations on biopsy materials from humans and experimental animals. As a first step, an attempt was made in the current study to obtain baseline data (information) on the pathology of experimental (animal) leishmaniasis caused by *L. mexicana* isolated from the Pacific lowlands of Ecuador. Together with the ultrastructural study of the Ecuadorian forms of leishmaniasis reported previously (Bhutto *et al.*, 1992), the information reported here will provide a clearer understanding of this disease in Ecuador. The present report focuses mainly on the results of histopathological findings of the dermal lesions (ulcers) of hamsters infected experimentally and the visceral organs invaded by the parasite *L. mexicana*.

Materials and Methods

Animals:

Male golden hamsters, 100 to 150 g and 7 weeks of age, were used. The animals were fade a standard pellet diet and given water *ad libitum*.

Parasites and mode of infection:

Parasites were isolated from an Ecuadorian leishmaniasis patient and maintained *in vitro* for several months before animal inoculation. They were identified as *L. mexicana* (MHOM/EC/INH690) using isoenzyme electrophoresis by Dr. T. Agatsuma, Department of Parasitology, Kochi Medical School, Japan. The promastigotes of log phase were inoculated to the nose and hind footpads of 10 hamsters. Each hamster received 1×10^7 promastigotes. The development of lesions was observed and recorded periodically during infection.

Histopathology:

At about 6 months after post-infection the animals were sacrificed and specimens were taken from central and peripheral parts of the lesions. Specimens were also taken from the liver and spleen and divided into two parts. One part was fixed in 10% formalin, from which paraffin sections were made and 5 μm sections were stained with hematoxylin-eosin. For the differentiation of mast cells, sections were also stained with toluidine blue (pH 5.0).

Electron Microscopy:

The other part of autopsy materials was cut into 2-3 mm pieces and fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). The tissues were then washed with 0.1 M cacodylate buffer and post-fixed in 2% osmium oxide for 2 hr. After dehydration in different concentrations of alcohol, specimens were embedded in epon 812. One micron semi-thin sections were cut with a glass knife on an LKB ultrotome and stained with toluidine blue. Ultra-thin sections were cut with a diamond knife, stained with lead citrate and uranyl acetate, and examined under a JEM 1200 EX electron microscope (JEOL, Japan).

Results

Almost all the hamsters inoculated with *L. mexicana* promastigotes developed erythematous lesions at the site of inoculation. The lesions gradually changed into nodules,

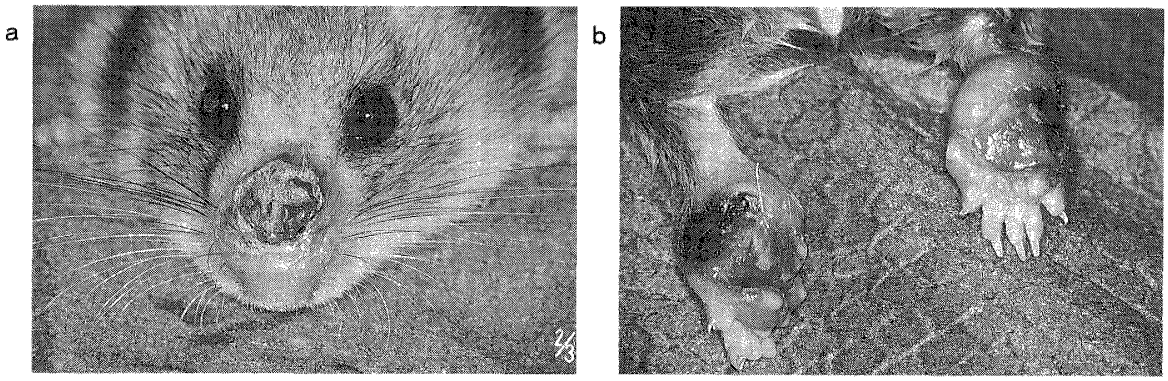


Figure 1 The inoculated sites of the golden hamsters infected with *L. mexicana* isolated from Ecuadorian patient. Shallow ulcers are visible on the (a) nose and (b) both hind footpads.

ultimately resulting in ulcers. At the time of autopsy, the inoculated sites of the nose and both hind footpads were observed as swollen and large ulcerations (Figs. 1a, b). The ulcers were covered with thick crusts. There was no metastasis or nodule formation on other areas of the body surface.

Light microscopic findings:

No significant epidermal changes were seen in either the nasal or the footpad sections. The epidermis was intact and thin at the center of the lesion, and crusts and parakeratosis were present in some parts of the specimens. In the dermis, histiocytes were predominant in the sections, and most of these contained numerous amastigotes in the large vacuoles, particularly at the central part of the lesions (Figs. 2a, b). Huge numbers of lymphocytes accumulated around the parasitized macrophages in the peripheral part of the lesions (Figs. 3a, b). Neutrophils, eosinophils and plasma cells were also seen throughout the specimens. Many mast cells were observed in the upper dermis in both the nasal and footpad sections (Figs. 4a, b). Mast cells were located in the areas of the dermis free from parasites but absent

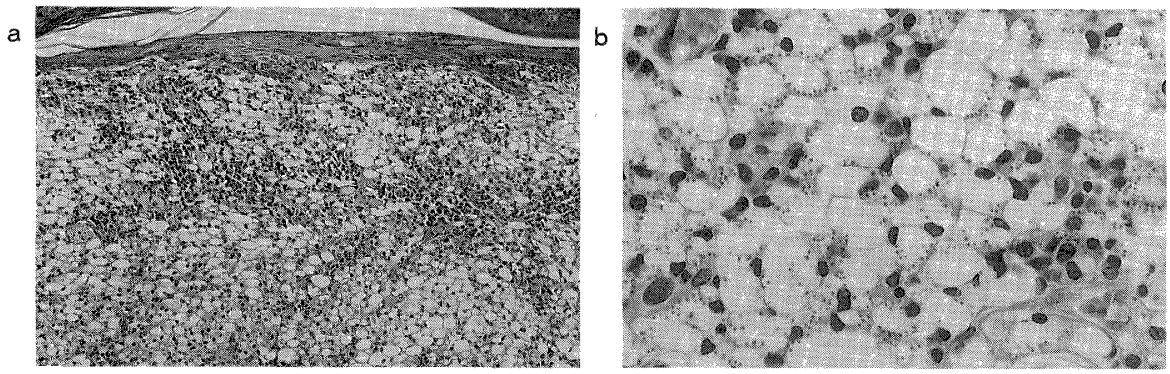


Figure 2 a, Light photomicrograph of formalin embedded section from the nose of a golden hamster infected with *L. mexicana,* showing the extensive infiltration of histiocytes and the formation of parasitophorous vacuoles. b, Many parasites invaded by macrophages are visible inside the parasitophorous vacuoles. (H & E stain ×40, ×160)

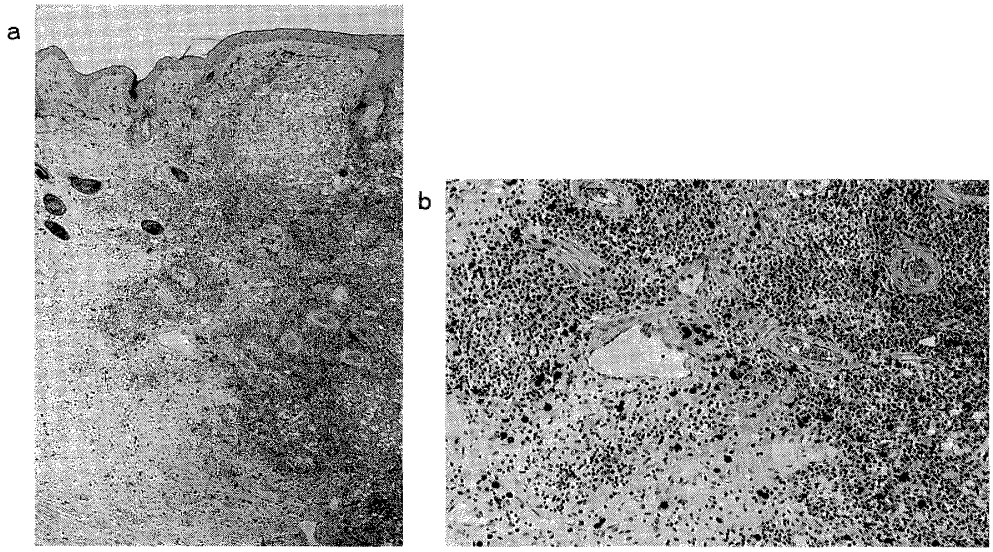


Figure 3 a, Footpad section, showing the presence of large number of lymphocytes and the parasitized macrophages. b, high magnification of 3a, showing the perivascular infiltration of lymphocytes. (H & E stain ×16, ×40)

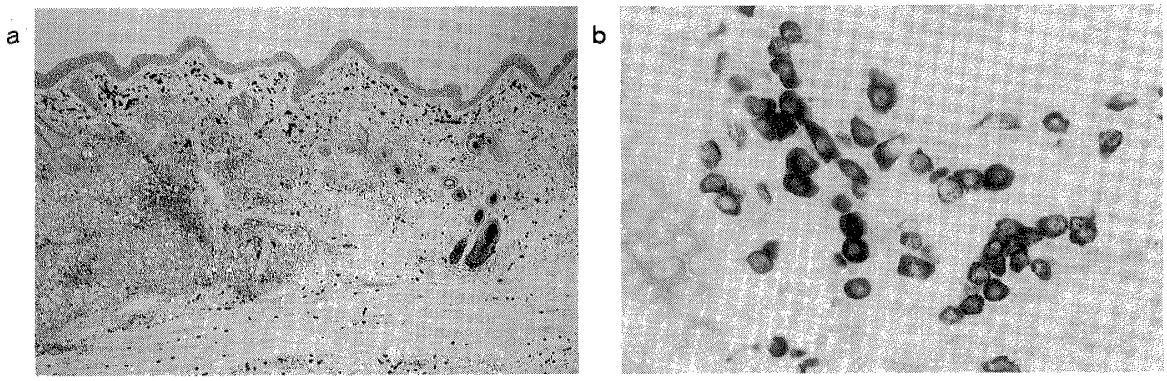


Figure 4 a, Huge number of mast cells in the upper and lower dermis. b, High magnification of 4a. (Toluidine blue stain ×16, ×160)

in the areas where macrophages were occupied with parasites. Focal necrosis was also recognized in the deep dermis.

In the liver and spleen sections, it was difficult to find the parasites because of the very small number.

Electron microscopic findings:

The epidermis of inoculated sites was free from the invasion of parasites. In the dermis, parasites were found both intracellularly and extracellularly. Amastigotes in the cytoplasm of macrophages were located either inside or outside vacuoles (Figs. 5 and 6). More than one vacuole was seen in most of the macrophages and large numbers of amastigotes were present in most of the vacuoles. Some parasite-free vacuoles were also seen, and these connected

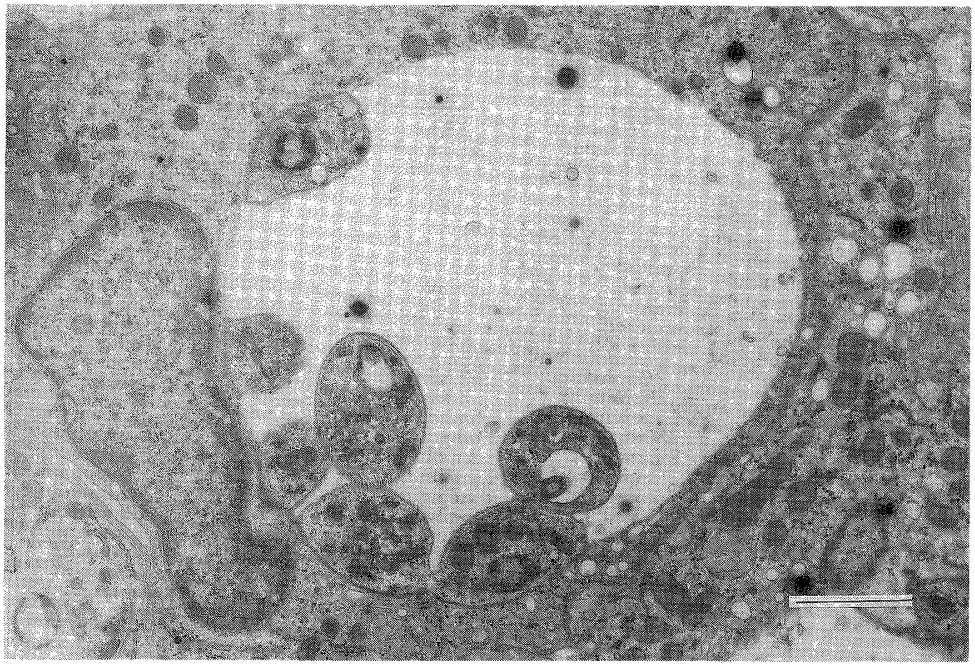


Figure 5 Electron micrograph. A group of *L. mexicana* parasites are evident inside the parasitophorous vacuole of macrophage. bar=2 μm

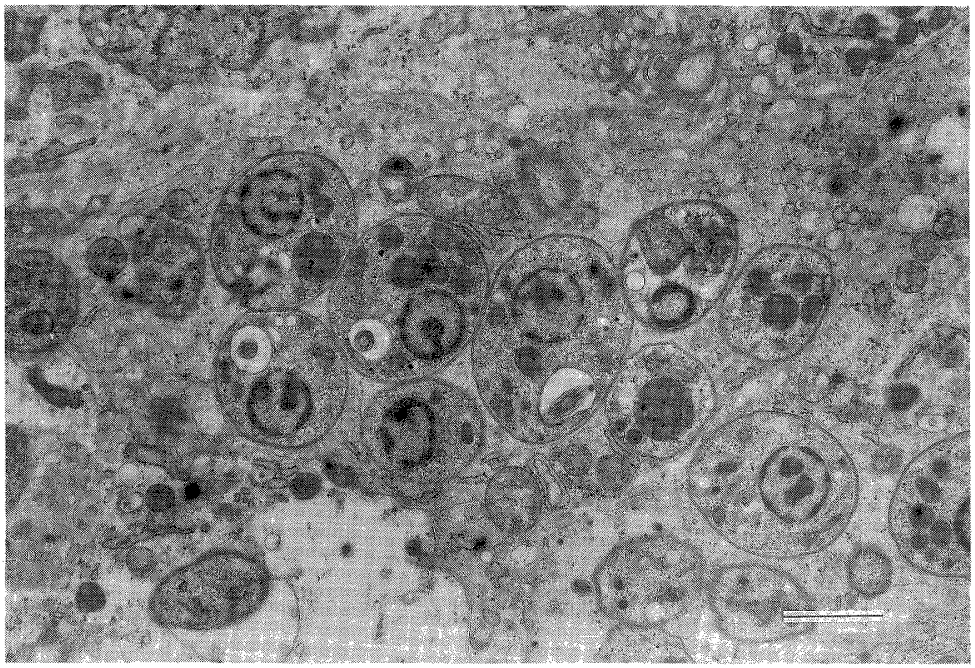


Figure 6 Figure showing the parasites inside the cytoplasm of macrophage without formation of vacuole. Mitochondria of the macrophage are also visible. bar=2 μm

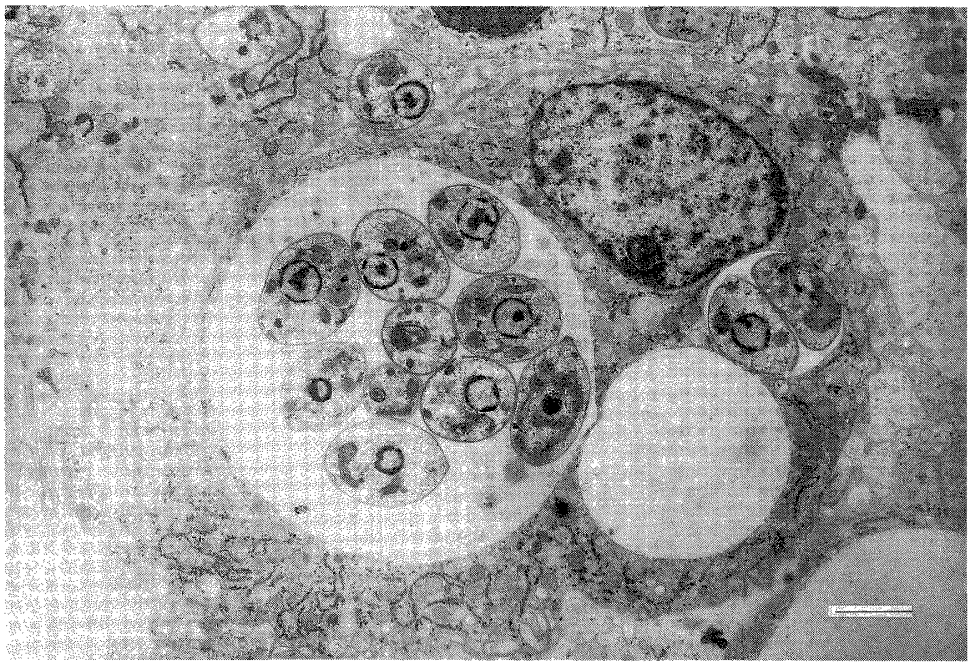


Figure 7 Various numbers of parasites with and without vacuoles. A small vacuole free of parasites is also visible near the large parasitophorous vacuole. bar=2 μm

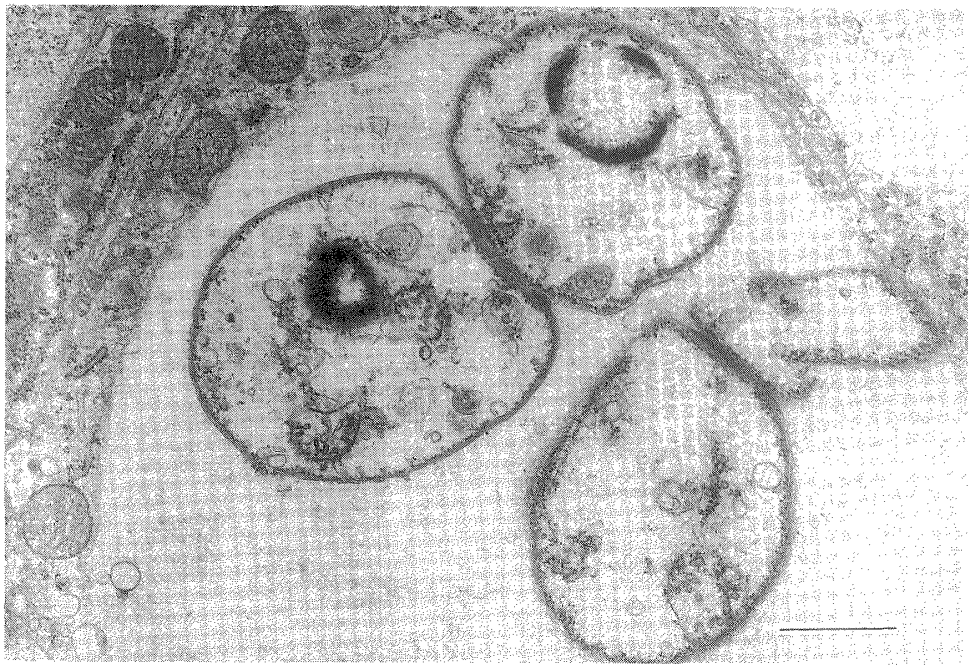


Figure 8 The degeneration of some *L. mexicana* parasites inside the macrophage of the liver. bar=1 μm

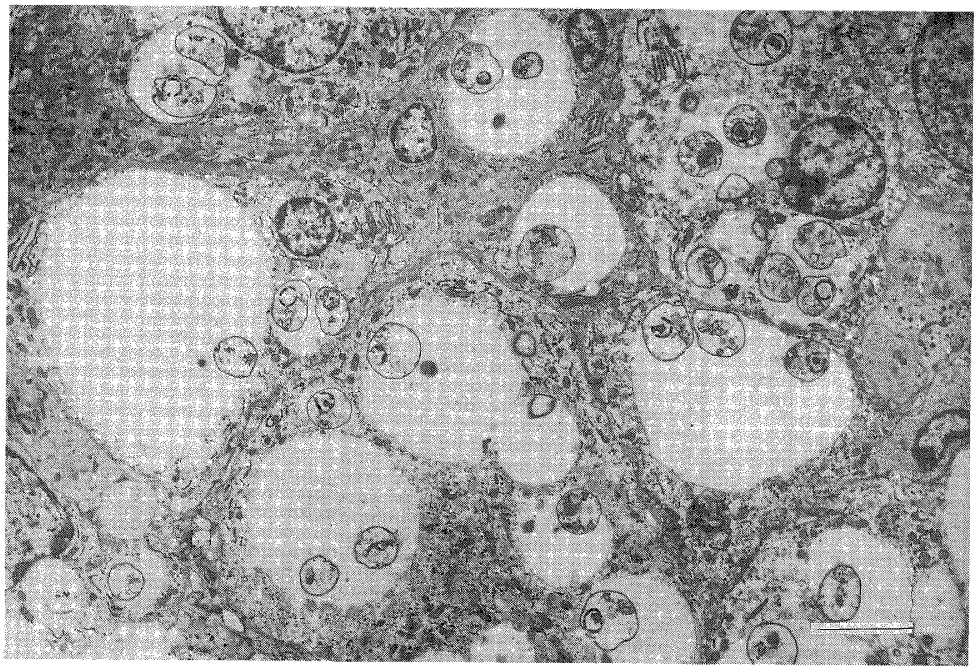


Figure 9 Electron micrograph of the section from the nose. Parasites are in various stages of degeneration. bar=5 μm

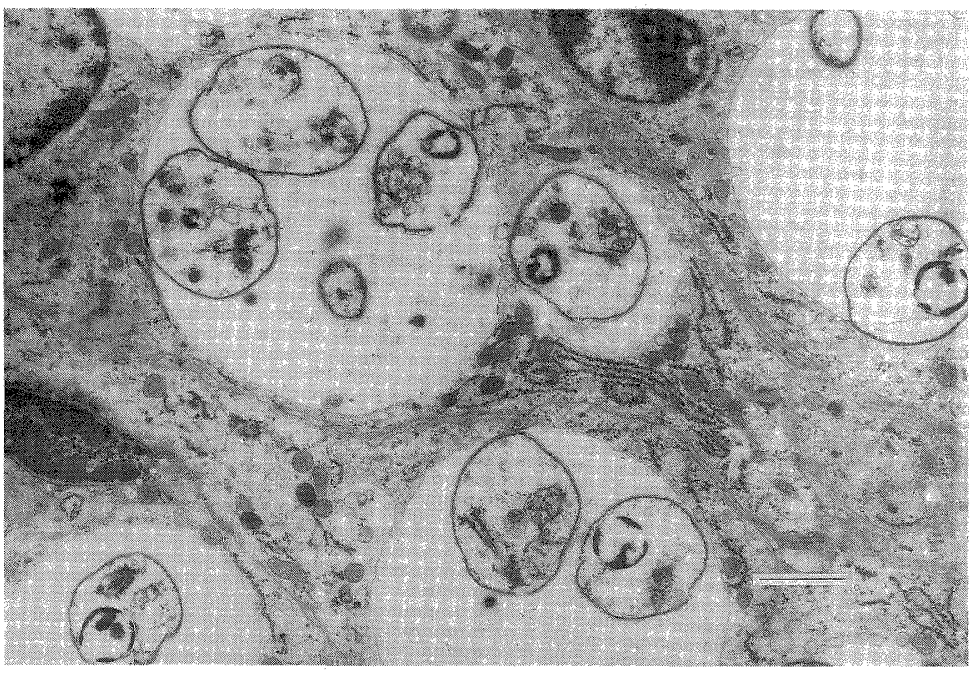


Figure 10 Degenerating parasites inside the parasitophorous vacuole of macrophage in the right footpad. bar=2 μm

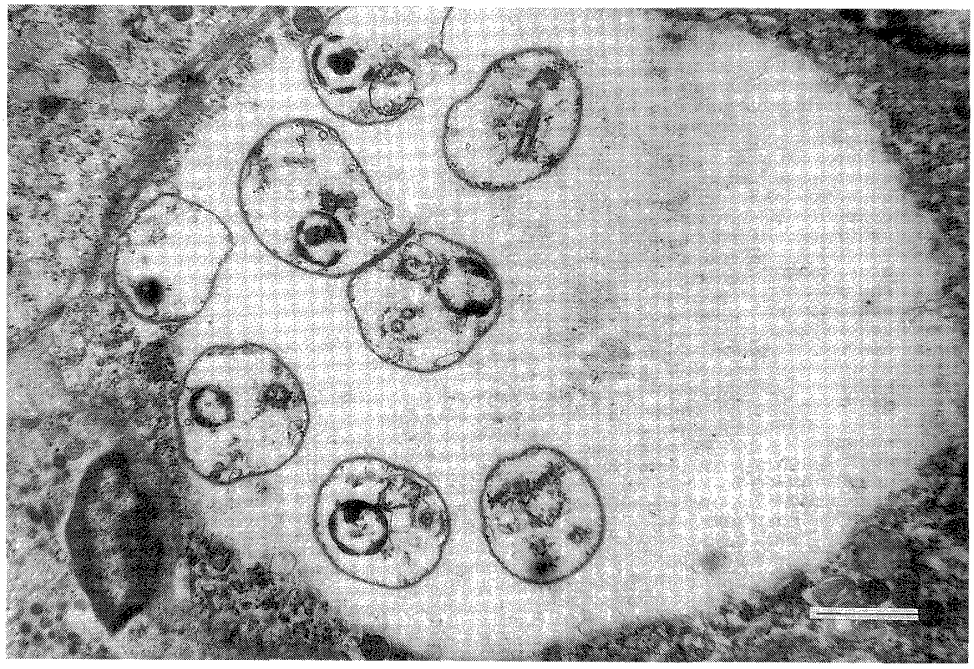
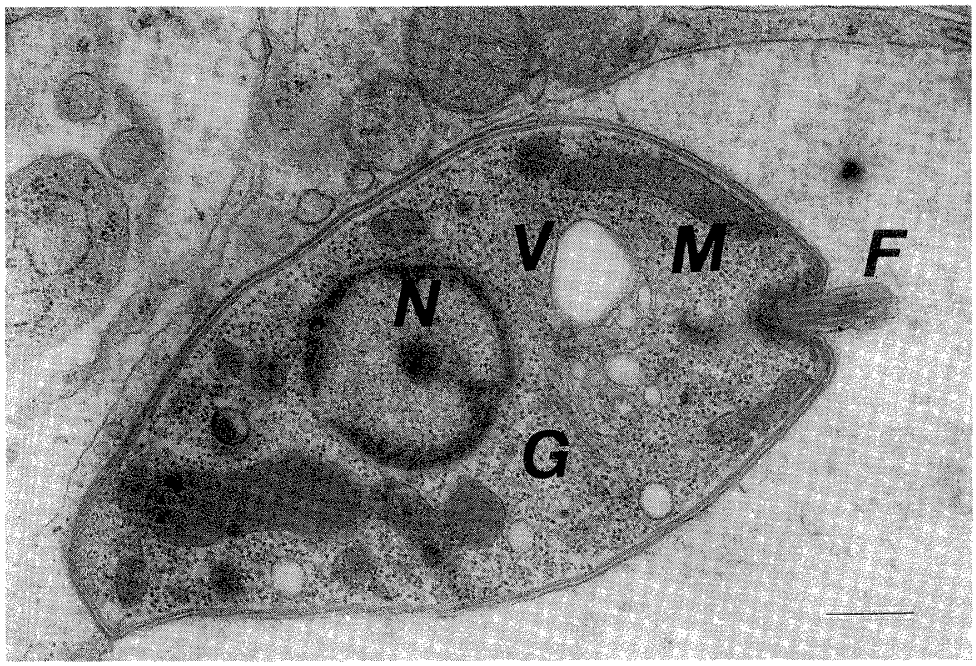


Figure 11 Section from left footpad. All the parasites are in various phases of degeneration inside the vacuole of macrophage. bar=2 μm

with parasitophorous vacuoles (Fig. 7). Parasites were neither inside the mast cells nor in the eosinophils or neutrophils.

Parasites were also found in the liver and spleen of hamsters infected experimentally with *L. mexicana* from Ecuador. They were located within the macrophages but very few in number. Regular degeneration of parasites was noted within macrophages inside or outside the vacuoles in all the cutaneous and visceral sections (Figs. 8, 9, 10 and 11). Morphologically, the normal amastigotes were rounded or elongated, surrounded by two layers of membranes and containing a rounded nucleus with a small nucleolus. The flagellum, flagellar pocket, kinetoplast, mitochondria, golgi apparatus, lysosomes, vacuoles and electron-dense granules in vacuoles were distinguishable (Figs. 12a, b).

a



b

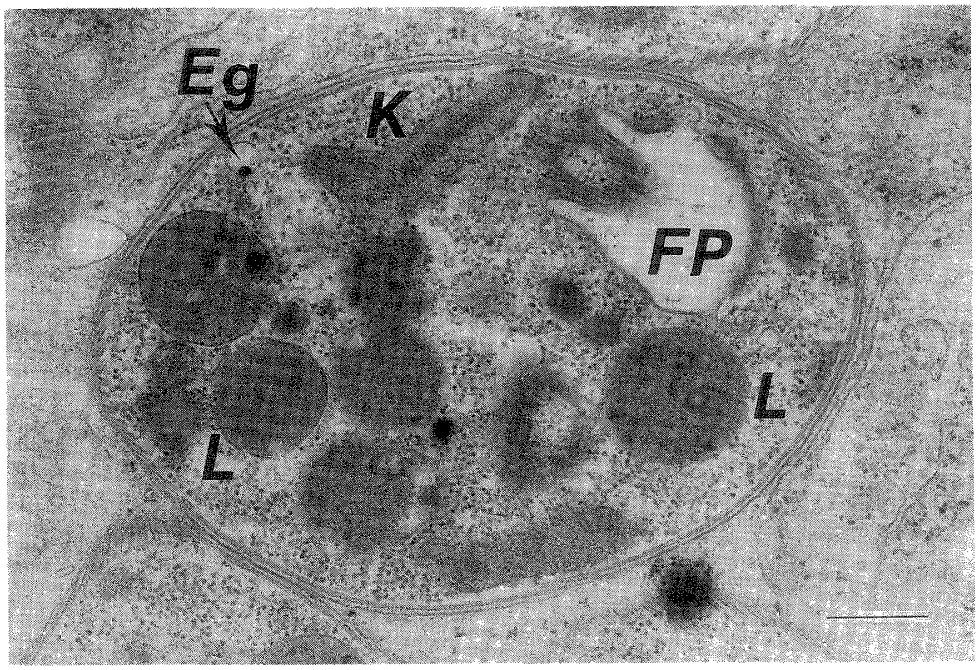


Figure 12a, b Ultrastructure of the *L. mexicana* parasite. F=flagellum, FP=flagellar pocket, K=kinetoplast, M=mitochondria, N=nucleus with small nucleolus, G=golgi apparatus, L=lysosomes, V=vacuoles and Eg=electron-dense granules inside the vacuole. bar=500 nm

Discussion

After the autopsy of hamsters 6 months after inoculation with *L. mexicana,* we observed shallow ulcers covered by thick crusts over the nose and both hind footpads in all the animals, confirming the finding that the hamster is a good animal model for *L. mexicana* infection (Wilson *et al.,* 1979; Bretana *et al.,* 1983).

An understanding of the relationship between *L. mexicana* and the cells of the host Ecuadorian patient is essential to the elucidation of the pathogenesis of the disease in that country. Observations of our specimens revealed that once the parasite is phagocytized by macrophage it is enclosed by phagolysosome. Then, the phagolysosome starts to distend and inside it multiplication of parasites takes place (Berman *et al.,* 1979).

With regard to the development of experimental cutaneous lesions, Bretana *et al.* (1983) proposed 1) that toxic factors from the amastigote inhibit the action of lysosomal enzymes; 2) that this causes the phagolysosome to become enlarged and new vacuoles to be invaded with parasites; 3) that extensive enlargement of the vacuoles and certain other factors cause the rupture of cell membranes; and 4) that repetition of this process results in ulceration at the sites. From the observations of many stained specimens in this study, we concluded that a similar process of ulceration takes place in hamsters infected with *L. mexicana* from Ecuador.

In the present light microscopic sections, large numbers of *L. mexicana* amastigotes inside the parasitophorous vacuoles were observed in the nose and footpad sections but it was difficult to find the amastigotes in the liver and spleen sections. The amastigotes in the liver and spleen were confirmed in ultra-thin sections but the number was limited, indicating the active multiplication of *L. mexicana* (dermatophilic) parasites in the inoculated sites (nose and footpads) but not in the liver and spleen. These findings suggest that although *L. mexicana* inoculated to the nose and footpads of hamsters can disseminate from the cutaneous sites to the liver and spleen, they do not replicate in these organs.

Similar results have also been presented by Hill (1988), who showed the possibility of dissemination of *L. amazonensis* to the liver and spleen in two different strains of mice. However, the metastasis (visceral dissemination) was not observed in C3H mice infected with *L. mexicana* (Grimaldi *et al.,* 1980). When the five different strains of mice were infected with *L. mexicana,* different responses were observed including the appearance of metastatic lesions in the tail of BALB/C mice (Perez *et al.,* 1979). Recently, *L. amazonensis,* well known as a causative agent of human cutaneous leishmaniasis, was isolated from the bone marrow of a Brazilian patient with visceral leishmaniasis (Barral *et al.,* 1986). With regard to *L. mexicana* infection, therefore, visceral dissemination may be observed in humans in future examinations as it was in the present animal (hamster) model.

There is controversy about the role of the macrophage in killing parasites intracellularly. Previously, we found the degeneration of parasites inside the macrophages in human specimens (Bhutto *et al.,* 1992). In the present study, large numbers of parasites also showed degeneration within macrophages inside and outside the vacuoles in all of the cutaneous and visceral specimens. This indicates the active role of macrophages in the destruction of parasites intracellularly, although the destruction mechanism is unknown. Intracellular degeneration of parasites inside macrophages has been reported in other human cases (Sandbank, 1976) and experimental works (Mauel *et al.,* 1978). On the other hand, however,

Bretana *et al.* (1983) showed that macrophages are incapable of destroying the parasites intracellularly.

Immunocytochemical and electron microscopic studies in animals have suggested that T-cells provide lymphokines (cytokines) that can activate the host macrophage to destroy the parasites intracellularly, or that T-cells play a cytotoxic role, killing the infected macrophages and helping to destroy the liberated extracellular parasites (McElrath *et al.*, 1987). Sypek *et al.* (1984) believed that intracellular destruction of *L. tropica* can take place only by the direct cell contact mechanism between lymphocytes and macrophages. Moreover, Murray *et al.* (1982) demonstrated the killing of *L. donovani* amastigotes inside macrophages by the oxygen dependent mechanism and concluded that non-activated phagocytes may display effective microbicidal activity against intracellular parasites utilizing the oxygen dependent mechanism.

From the present hamster and *L. mexicana* model, we propose that some unknown factor/factors (as discussed above) under host immune mechanism(s) play an important role and promote the destruction of parasites inside the macrophages, and therefore that effective killing of the parasites finally might result in healing of the lesions. However, the precise conditions and mechanisms of the destruction of parasites were not elucidated in the present study. In our specimens, severe degeneration of amastigotes in large numbers of macrophages may represent a forward step in the healing process and may allow us to suggest a possible mechanism in the self-healing of the disease. We did not, however, observe the complete healing of the lesions. Our experimental study using hamsters infected with *L. mexicana* isolated from an Ecuadorian patient is a model of classic self-healing of the cutaneous form that adds to our understanding of the mechanism of the pathogenesis of Ecuadorian leishmaniasis. On the basis of the results obtained, we conclude that macrophage can destroy the parasites intracellularly under certain mechanism(s) and play an effective and major role in the spontaneous healing of the disease.

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実験的リーシュマニア症
—エクアドルで分離された *Leishmania mexicana* 感染ゴールデンハムスターの組織学的検討—

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実験はゴールデンハムスターに，*Leishmania mexicana* を感染させて行った。接種後6カ月目に屠殺した時，接種部位は厚い痂皮を付す浅い潰瘍となっていた。躯幹の他の部位には，病変を認めなかった。接種部位（鼻，足蹠）標本の病理組織学的所見は，多数の amastigotes (AG)，組織球，リンパ球の広範な浸潤が見られ，好中球，好酸球，プラズマ細胞を軽度混在していた。真皮の肉芽腫性病変の上下では，マスト細胞の出現が著明であった。AG は病巣中央部のマクロファージにある，大きな空胞内に存在していた。また電顕的には，肝臓，脾臓にも AG が認められたが，皮膚に比べると数は少なかった。皮膚，および内臓のマクロファージ内で観察された寄生虫の破壊は，この細胞の貪食作用によるものと思われた。

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Short communication

SERUM C-REACTIVE PROTEIN (CRP) VALUES OF PATIENTS INFECTED WITH *TAENIA SAGINATA*

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Elevated serum C-reactive protein (CRP) values are identified in patients with infections, some tumors or various types of tissue destruction, and clinical measurement of serum CRP is valuable as a screening test for organic diseases and as a sensitive index of disease activity and response to therapy in the disease mentioned above (Pepys, 1981). But, to our knowledge, CRP has been given but scant attention in cases of tapeworm infection. Human infection with *Taenia saginata* is uncommon in Japan and clinical manifestations of this tapeworm infection are mild or symptomless, but infection with *T. saginata* is an important parasitic diseases because of its world wide distribution.

From April 1987 to March 1991, we treated 5 patients with *T. saginata* infection. All of the patients were Japanese males aged 23 to 66 years old. None of them had a previous history of the tapeworm infection. All of the infections were asymptomatic, except for the discomfort and embarrassment occasioned by the crawling of proglottids from the anus. The place of contraction of 3 patients was Ethiopia, 1 patient Japan and 1 patient unknown. All of the patients expelled a single *T. saginata* with intraduodenal gastrographin injection or oral administration of paromomycin sulphate. Before the therapy, serum CRP levels were measured with a Behring Nephelometer Analyzer with CRP-Latex reagent kit.

The serum CRP value of all patients was under 0.3 mg/d*l* and this value was in normal range. The results are shown in Table 1.

The results reported here show that CRP values of patients infected with a single *T. saginata* are in the normal range. It has been reported that CRP was found in a human liver

Table 1 Pre-treatment serum CRP value in patients infected with a single intestinal *T. saginata*

Case	Age (years)	Sex	Place of contraction	CRP (mg/d*l*)
1	34	M	Ethiopia	<0.3
2	32	M	Japan	<0.3
3	23	M	Ethiopia	<0.3
4	26	M	Ethiopia	<0.3
5	66	M	unknown	<0.3

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culture but not in bone marrow, spleen, thyroid, mammary gland or thoracic duct lymphocytes in culture (Hurlimann *et al.*, 1966) and that interleukin-1 (IL-1) and interleukin-6 (IL-6) both stimulated liver synthesis of CRP and that probably IL-6 played a key role in stimulation of CRP (Hazenberg *et al.*, 1988). The results of these reports indicate that the liver is an important organ for producing CRP and that the production is stimulated by interleukins stimulated by tissue damage. *T. saginata,* whose head has four suckers lives by attaching to the small intestinal wall. Our results suggest that single infections with *T. saginata* may not induce interleukins sufficient to increase the CRP value. It is thought that injury to the intestinal wall by a single *T. saginata* is so mild that intestinal infection by the tapeworm does not stimulate production of CRP in the liver to clinically detectable levels. We think that if an elevated serum CRP value is observed in an asymptomatic person infected with a *T. saginata,* other abnormal accompanying conditions such as neoplasma or collagen disease should be considered.

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無鉤条虫感染症における血清CRP値

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単数寄生の無鉤条虫感染症患者5人で，治療前血清CRP値を測定した。症例は全員日本人男性で，片節の排出時の不快感以外に症状はなかった。結果は全例で<0.3 mg/dlであり，正常範囲にあった。このことから，単数の無鉤条虫の腸管寄生は，血清CRP値を上昇させる原因とはならないと考えられた。腸管の単数の無鉤条虫感染症で血清CRP値の上昇を認めた場合，合併症の検索が必要と考えられる。

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