Visceral Leishmaniasis in a Dog: Clinical, Hematological and Pathological Observations

L. Tryphonas, Z. Zawidzka, M. A. Bernard and E. A. Janzen*

ABSTRACT

Visceral leishmaniasis was diagnosed in a dog that had been living with his owners in Spain for two years. Clinical diagnosis was somewhat delayed as the disease is largely unknown to Canada and was manifested by a nonresponsive anemia which was not easily explained on peripheral blood evaluation alone, and concomitant interstitial nephritis. On post mortem examination splenomegaly was the main gross pathological finding. Light microscopic examination of bone marrow aspirates and subsequent electron microscopic examination of splenic and hepatic tissues revealed numerous Leishman-Donovan bodies in cells of the reticuloendothelial system. Parasitized reticuloendothelial cells were seen singly or forming granulomata. These latter did not contain giant cells and were confined mainly to the liver and spleen, being sparse and single in the first but extremely numerous and coalescing in the latter. Accumulation of intrafollicular hyaline material was seen in a small number of splenic follicles. Leishman-Donovan bodies on electron microscopic examination had a trilaminar periplast, a large round nucleus with heavy blocks of marginated chromatin and two nucleoli, a short flagellum and a kinetoplast. Lymph nodes and bone marrow had numerous parasitized macrophages but no granulomata. Leishman-Donovan bodies were not detected in the lungs and kidneys both of which exhibited a chronic interstitial reaction. The comparative hematological profile as well as the importance of bone marrow and electron microscopic examinations of the spleen and liver in diagnosis are discussed. The potential public health hazard of leishmaniasis to North America and particularly to Canada is considered.

*Pathology Section, Research Laboratories, Health Protection Branch, Department of National Health and Welfare, Tunney's Pasture, Ottawa, Ontario K1A 0L2 (Tryphonas and Zawidzka) and Alta Vista Animal Hospital, 1814 Bank Street, Ottawa, Ontario K1V 7Y6 (Bernard and Janzen).

Submitted November 14, 1975.

RÉSUMÉ

Les auteurs ont diagnostiqué un cas de leishmaniose, chez un chien qui avait passé deux ans en Espagne avec ses maîtres. L'établissement tardif du diagnostic clinique de la condition s'explique par le fait que la leishmaniose n'existe pratiquement pas au Canada et qu'elle se manifeste par une anémie réfractaire qu'on s'expliquait difficilement en ne se basant que sur des examens hématologiques; de plus, le chien souffrait simultanément de néphrite interstitielle. Lors de la nécropsie, la principale lésion consistait en une splénomégalie. L'examen microscopique d'aspirations de moelle osseuse et des examens ultérieurs du foie et de la rate, à l'aide de la microscopie électronique, révélèrent la présence de plusieurs corps de Leishman-Donovan dans les cellules du système réticulo-endothélial. Les cellules parasitées se présentaient individuellement ou sous la forme de granulomes. Ceux-ci ne contenaient pas de cellules géantes et se confinaient surtout dans le foie et la rate; rares et individuels dans le foie, ils étaient par ailleurs très nombreux et fusionnés, dans la rate. La pulpe blanche splénique contenait un peu de substance hyaline. La microscopie électronique révéla que les corps de Leishman-Donovan possédaient un périplaste trilaminaire, un gros noyau rond, pourvu de points denses périphériques de chromatine et de deux nucléoles, un flagelle court et un cinétoplaste. Les ganglions lymphatiques et la moelle osseuse contenaient plusieurs macrophages parasités, mais pas de granulomes. Les auteurs ne décelèrent pas de corps de Leishman-Donovan dans les poumons ou les reins; ces organes présentaient cependant une réaction proliférative interstitielle. Ils commentent le profil hématologique comparatif, ainsi que l'importance diagnostique de l'étude de la moelle osseuse et de l'examen au microscope électronique de la rate et du foie. Ils disent aussi quelques mots sur le danger éventuel de la leishmaniose pour la santé publique en Amérique du Nord, particulièrement au Canada.

INTRODUCTION

Leishmaniasis caused by *Leishmania donovani* is a geographically restricted, arthropod transmitted protozoan zoonosis affecting among other mammals dogs and man (11, 27, 28, 29). It is a disease of the reticuloendothelial system (RES) and is best known to be enzootic and endemic in parts of the Americas, Near East as well as in peri-Mediterranean countries from where it is occasionally imported into countries known to be free of the parasite (13, 15, 16, 18, 27, 28, 29). Its true distribution however is much more world-wide (9, 25, 31, 33). Although several forms of the disease have been described, visceral or systemic leishmaniasis (Kala-azar) is the severest and commonest form in both man and dog (7, 11, 27).

The importance of canine leishmaniasis lies in the well established fact that, in enzootic areas where sandfly vectors (species of *Phlebotomus)* and *Leishmania donovani* co-exist, dogs are an important reservoir host from which the *Leishmania* can easily spill over to the human population. It has been indicated however that in the case of man the disease can be transmitted also by contact (27).

Diagnosis of leishmaniasis has been traditionally based on the demonstration of the organism and/or lesions by histopathological, culture, animal inoculation and serological techniques (1, 18), but it is often delayed in disease free areas. Electron microscopic examination is a recent adjunct to the armamentarium of diagnostic methods (4, 6).

The purpose of the present report is to describe the clinical, hematological and pathological observations on imported canine leishmaniasis, discuss the significance of bone marrow evaluation and electron microscopic examination of the hemopoietic and other tissues in the diagnosis of cases in which circumstances preclude biological tests and compare existing similarities and dissimilarities between human and canine leishmaniasis.

HISTORY AND CLINICAL FINDINGS

On October 2, 1972 a six year old. spayed. black Labrador Retriever dog was presented with listlessness and the complaint of pain in the left hind leg, and slow but progressive loss of body weight over a period of two months. Past history revealed that the dog had travelled extensively, having had a fracture repaired in Québec City in 1967, being spayed in Dartmouth, Nova Scotia in 1968 and having been in the village of Mijas, Spain from September 1970 to September 1972.

Clinical examination revealed fair bodily condition, dull hair coat, slight pallor of visible mucous membranes and swelling of the left femorotibial joint with crepitation on flexion. The results of fecal examination for parasites were negative. Roentgenograms revealed osteoarthrosis of the left coxofemoral and femorotibial joints. Urine analysis and peripheral blood evaluation results were interpreted to indicate that the dog had normochromic, normocytic anemia and nephropathy (Table I). Hematinic and supportive treatment was prescribed and the dog was released. Subsequently the dog was admitted repeatedly for further examination including exploratory laparotomy and treatment as she was not responding satisfactorily and developed new signs such as cough, bilateral iritis and uveitis, weakness, anorrhexia, vomiting, loss of weight and persistent anemia (Table II).

On November 14, blood serum analysis revealed elevated total protein with hypoalbuminemia, hyperglobulinemia and a markedly lowered A-G ratio (Table III). On November 17, leishmaniasis was diagnosed when numerous *Leishmania* organisms were detected in bone marrow smears (Table IV). Antimonic treatment, instituted promptly, was partially successful. The dog's health improved considerably with some remissions for approximately five months but deteriorated rapidly during the last five days of life. The dog died on April 16, 1973.

MATERIALS AND METHODS

Blood samples were taken in 15% EDTA tripotassium salt as an anticoagulant at a concentration of 0.01 ml/1.0 ml of blood. Erythrocyte (RBC) and total leukocyte (WBC) counts as well as mean corpuscular

TABLE I. Canine Leishmaniasis. Results of Repeated Urine Examinations over a Period of Three Months

	Dates			
	2 October 1972	7 November 1972	14 November 1972	3 January 1973
Analysis				
Specific gravity	1037	1050	1030	1054
pH	6.5	6.0	6.5	7.0
Protein mg/dl	300	300	100	300
Glucose, bilirubin, ketones	Neg	Neg	Neg	Neg
Microscopic Examination				
RBC/h.p.f.[a]	none seen	few	few	few
WBC/h.p.f.	occasional	some	few	few
Casts	none seen	occasional granular	none seen	few granular
Epithelial cells	some	some	occasional	few

[a]h.p.f. = high power field

TABLE II. Evaluation of Peripheral Blood During the Course of Canine Leishmaniasis

	16 November 1972	19 December 1972	2 March 1973	12 April 1973
Erythrocytes x 10^{12}/1	3.22	3.12	3.27	2.08
Hemoglobin gms/dl	8.3	8.1	8.6	5.1
Hematocrit 1/1	0.25	0.23	0.24	.041
MCV fl	74	74	74	67
MCH pg	26	26	26	24
MCHC g/dl	33	35	36	36
Reticulocytes % of 3000 cells	—	0.8	—	—
Total leukocytes x 10^9/1	13.300	7.200	5.300	7.000
Basophils x 10^9/1	0 (0.0)[a]	0 (0.0)	0 (0.0)	0 (0.0)
Eosinophils x 10^9/1	0.067 (0.5)	0.400 (5.5)	0.610 (11.5)	0 (0.0)
Neutrophils (bands) x 10^9/1	0.467 (3.5)	0.220 (3.0)	0.210 (4.0)	0.175 (2.5)
Neutrophils (segm) x 10^9/1	10.100 (76.0)	5.100 (71.0)	2.790 (52.5)	5.950 (85.0)
Lymphocytes x 10^9/1	0.933 (7.0)	0.865 (12.0)	1.030 (19.5)	0.280 (4.0)
Monocytes x 10^9/1	1.730 (13.0)	0.615 (8.5)	0.660 (12.5)	0.595 (8.5)
Number of cells counted	200	200	200	200
Nucleated red cells/200 WBC	none	1 metarubricyte	none	none

[a]Brackets = % values

volume (MCV) and hemotocrit (HCT) values were obtained using a Coulter electronic counter model F_n and MCV/HCT accessory.[1] Hemoglobin (HGB) was determined by a cyanmethemoglobin method using a Coulter hemoglobinometer. Blood smears, for differential counts and morphological assessment; and bone marrow preparations, obtained under general anesthesia from the femur and/or iliac crest, were both stained with Leishman's. Reticulocytes were supravitally prestained with new methylene blue and then counterstained also with Leishman's.

Hematoxylin and eosin (H&E) stained sections and wet 10% formalin fixed tissue

TABLE III. Canine Leishmaniasis. Selected, Blood Serum, Biochemical Parameters (14 November 1972)

Parameters	Result	Normal Values
Cholesterol mg/dl	350.00	125 — 250
Calcium mg/dl	10.00	9 — 11.5
Inorganic phosporus mg/dl	6.10	2.5 — 5.0
Total bilirubin mg/dl	1.80	0 — 0.6
Total protein mg/dl	9.50	4.9 — 7.9
Albumin gm/dl	2.50	3 — 4.8
Globulins gm/dl	7.00	1.3 — 3.2
A/G	0.35	> 1
Uric acid mg/dl	2.50	0 — 1
Urea nitrogen mg/dl	5.00	10 — 20
Glucose mg/dl	40.00	55 — 99
LDH (Wacker units)	>350	>260
Alkaline phosphatase (King Armstrong Units)	12	>10.4 ± 3SD
SGOT (Karmen Units)	150	20 — 35

[1]Coulter Electronics Inc., Hialeah, Florida.

from liver, spleen, bone marrow (femur), joint capsule and adjacent muscle, lungs, lymph node, and small intestine were available for examination. All tissues were processed by conventional methods and embedded in paraffin. Selected serial sections were stained with H&E, periodic acid Schiff reaction (PAS), Leishman, Giemsa, Jone's reticulin, periodic acid methenamine silver (PAMS), Prussian blue and Gridley fungus methods (17).

Formalin prefixed blocks of spleen and liver were immersed in osmium tetroxide, dehydrated through a graded series of methanol and embedded in araldite, Semithin sections stained with 0.5% toluidine blue in 1% borax and ultrathin sections stained with uranyl acetate and lead citrate were examined with a Siemens Elmiscope 101 electron microscope at 80 KV (21).

TABLE IV. Evaluation of Bone Marrow Aspirates During the Course of Canine Leishmaniasis (% values)

	17 November 1972	19 December 1972	19 January 1973	8 February 1973	13 April 1973
Total myeloid series	58.9	64.8	67.0	55.8	58.0
Blasts	2.0	1.8	0.8	1.0	1.0
Progranulocytes	3.4	4.0	2.8	3.0	4.2 (0.2)[a]
Myelocytes neutrophils	4.2 (0.3)	2.4 (0.2)	1.6 (0.4)	2.6 (0.2)	2.4 (0.2)
" eosinophils	0.8	0.6	1.0	1.0	0.0
" basophils	0.0	0.0	0.0	0.2	0.2
Metamyelocytes neutrophils	6.8	7.4	4.4	6.2	8.2
" eosinophils	0.5	0.2	1.0	1.2	0.0
" basophils	0.0	0.0	0.0	0.0	0.0
Bands	26.6	25.0	27.6	23.0	25.6
Mature neutrophils	13.7	21.0	25.8	14.2	15.0
Eosinophils	0.9	2.4	2.0	3.4	1.4
Basophils	0.0	0.0	0.0	0.0	0.0
Total erythroid series	6.8	22.6	26.2	32.8	23.0
Rubriblasts	0.1	0.4	0.4	0.4 (0.2)	0.2
Prorubricytes	0.7	1.0	2.2 (0.2)	1.4	1.8
Rubricytes	4.4 (0.1)	12.8 (0.6)	16.4 (0.8)	17.4 (0.8)	11.4 (0.4)
Metarubricytes	1.6	8.4	7.4	13.6 (0.2)	9.6
Undifferentiated cells	0.0	0.0	0.0	0.0	0.8
Lymphocytes	10.9	7.2	5.6	8.6	15.0
Plasma cells	13.8	3.6	0.8	1.6	1.4
R-E cells	9.3	1.6	0.4	1.0	1.8
Tissue basophils	0.0	0.0	0.0	0.0	0 0
Megakaryocytes	0 2	0.2	0.0	0.2	0.0
Osteoclasts	0.1	0.0	0.0	0.0	0.0
Number of cells counted	1000	500	500	500	500
Myeloid-erythroid ratio	9.6	2.9	2.6	1.7	2.5
R-E cells containing L-D bodies	All	3-4[b]	1[b]	none seen	1-2[b]
Cellularity	Normoplastic	IWE[c]	IWB	IWB	Normoplastic

[a]Brackets = % cells in mitosis
[b]Extensive search was required
[c]IWB = infiltrated with blood

RESULTS

Hematological Findings

Quantitation of erythrocytes and leukocytes, determination of HCT and HGB levels, and morphological assessment of erythrocytes, leukocytes and platelets revealed normocytic predominantly normochromic anemia, normal total WBC, relative and borderline absolute lymphopenia and moderate relative and absolute monocytosis. Morphologically all cellular elements, including platelets were normal.

Examination of bone marrow smears suggested cellular but not hyperplastic sample. The differential count indicated severely impaired erythropoiesis with normal morphology and maturation sequence of the erythroid series. Plasma cells and reticuloendothelial cells were increased in number.

The latter contained numerous round or oval 2-4 μm parasites having deeply staining nucleus and rod-like reddish kinetoplast (Fig. 1). Only parasites scattered free near ruptured cells could be studied in detail. They were identified as Leishman-Donovan (L-D) bodies. The myeloid series was morphologically and quantitatively normal while the myeloid erythroid (ME) ratio was markedly increased (Table IV).

Despite specific and supportive antianemic treatment peripheral blood erythroid values remained virtually unchanged until the last relapse prior to death. At this time the nonresponsive anemia had become even more severe. The total WBC count fluctuated between normal and borderline low throughout the duration of the disease. Relative neutrophilia persisted except for the occasion when both relative and absolute numbers fell below normal limits. Eosinopenia developed terminally. Absolute granulocyte values were otherwise within normal range. Relative and slight absolute lymphopenia progressed to a marked degree before death. Absolute numbers of monocytes, initially somewhat elevated, decreased and remained normal until death (Tables II and IV).

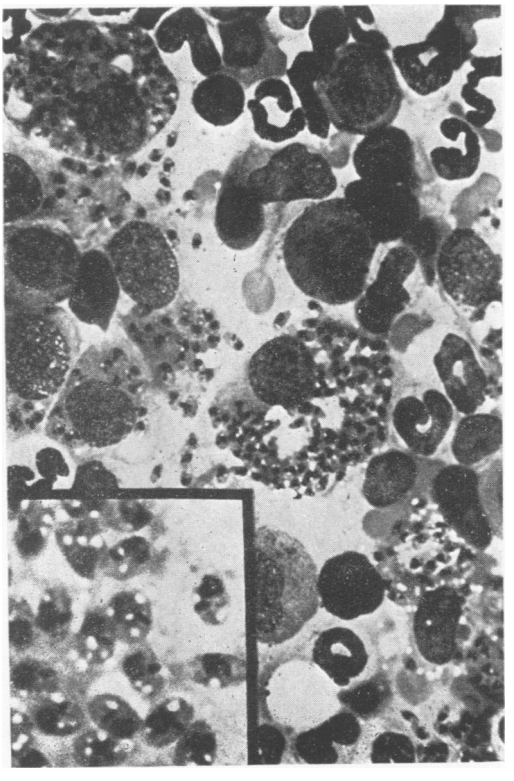

Fig. 1. Bone marrow smear. Macrophages with large numbers of L-D bodies. Insert: Kinetoplast and nucleus of L-D bodies are clearly discernible. Leishman stain. X865.

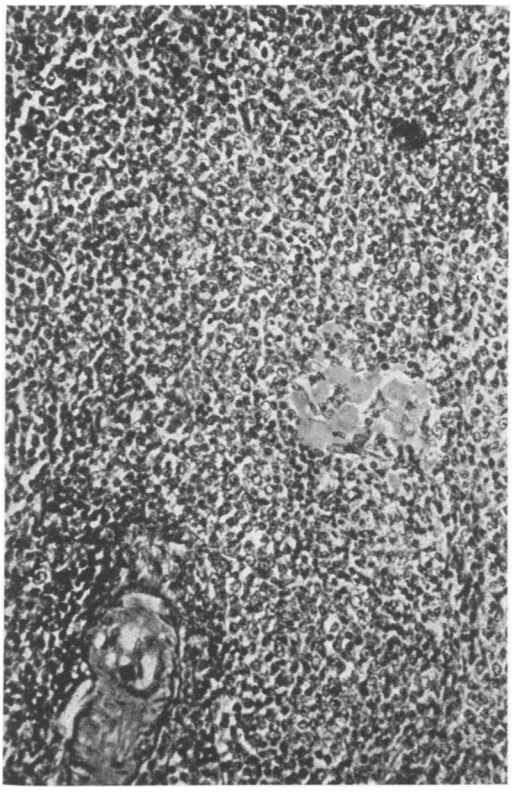

Fig. 2. Splenic follicle with dense cellularity and intrafollicular hyalinosis. PAS. X100.

Subsequent post-treatment bone marrow examinations revealed a drastic reduction in the number of reticuloendothelial cells few of which contained L-D bodies, normal percentage of plasma cells and increased erythroid activity (Table IV). Eosinophilic precursors were significantly reduced during the terminal relapse (Table IV).

Gross Findings

Moderate dehydration and emaciation were present. The spleen was thickened, meaty and had rounded borders. The overall size of the spleen reached at least twice that of normal. The liver was slightly enlarged and was moderately congested.

Light Microscopic Findings

The splenic trabeculae were prominent

while the white pulp consisted of dense enlarged lymphoid follicles, some with distinct germinal centers and several with intrafollicular hyalinosis (5) (Fig. 2). The red pulp was densely cellular and contained little blood (Fig. 3). The increased cellularity of the red pulp was due to a florid proliferation of macrophages that gave rise to granulomata of single cell type without giant cells (Fig. 5). The granulomata were often surrounded by a variably thick layer of lymphocytes, lymphoblasts, and plasma cells (Fig. 4). Proliferating histiocytes harboured in their cytoplasm numerous L-D bodies. These tended to be mostly round or occasionally C-shaped dark staining bodies infrequently surrounded by a halo (Fig. 5). Parasitized macrophages were ubiquitous except within lymph follicles where they were rarely seen. They accumulated also within the trabecular vessel walls and were prominent subendothelially, from where they were presumably discharged into the lumen (Fig. 6). Some parasitized macrophages harboured hemosiderin pigment, in which the presence of iron was confirmed with the Prussian blue reaction as well as with X-ray probe analysis in a Siemens Elmiscope 101 electron microscope. The splenic capsule was thickened and infiltrated with numerous parasite-laden macrophages.

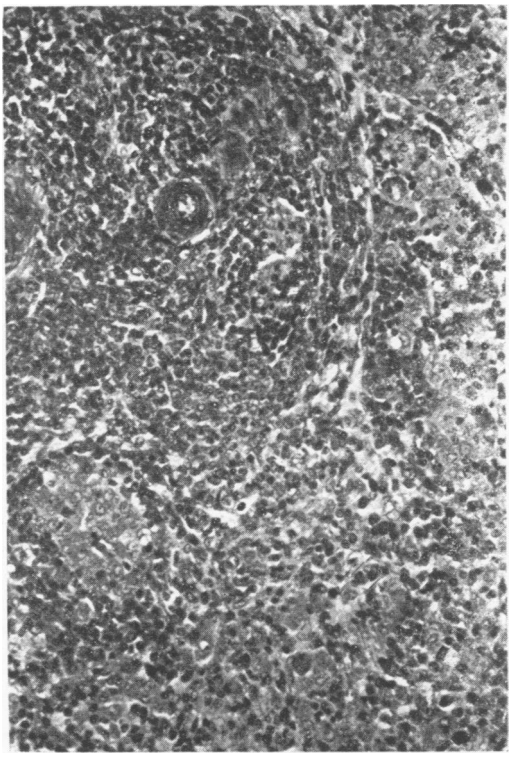

Fig. 4. Splenic granuloma (lower right) consisting of a dense accumulation of parasitized macrophages and surrounded by a thin rim of lymphocytes, lymphoblasts and plasma cells. Giant cells are absent. H & E. X100.

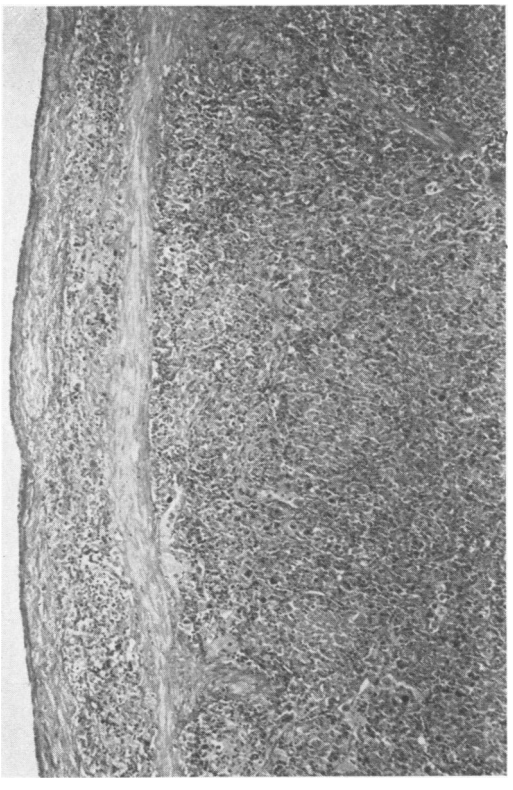

Fig. 3. Spleen. Thickened capsule and trabeculae with numerous granulomata in the red pulp resulting from hyperplasia of the RES. PTAH. X25.

The coarse architecture of the hepatic parenchyma was undisturbed. There was, however, noticeable centrilobular congestion and mild periacinar fatty degeneration. Close examination revealed enlargement of Kupffer cells and other histiocytes many of which contained numerous L-D bodies and/or hemosiderin, as well as a slight sprinkling of lymphocytes and plasma cells throughout the liver (Figs. 6 and 7a). Parasitized macrophages tended to form early granulomata. Larger granulomata caused compression atrophy of adjacent hepatic plates (Fig. 7b). Necrosis or proliferation of connective tissue and giant cells were absent from such granulomata. It was then concluded that an early granulomatous hepatitis was developing.

Granulomata were not detected in the lymph nodes, although histiocytes laden

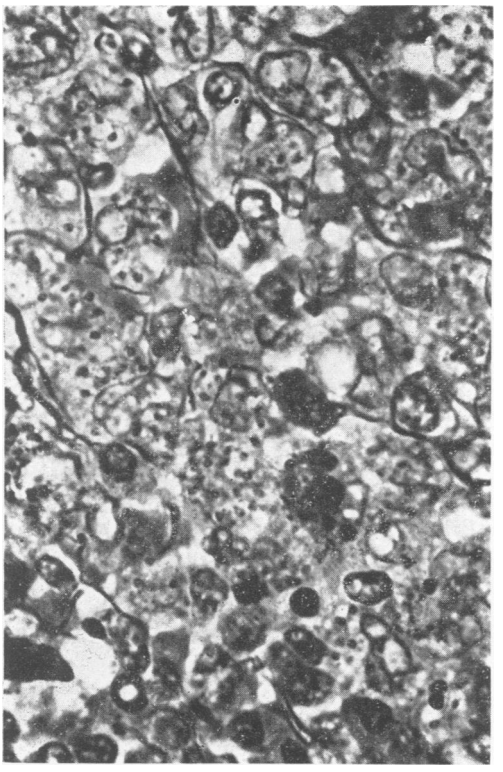

Fig. 5. Splenic red pulp with numerous heavily parasitized macrophages. PAMS. X925.

with L-D bodies were common in the trabeculae and less so in the sinuses.

Although the bone marrow had not fixed well, parasitized macrophages were not difficult to find. No further comments can be made however, on the type of reaction in the marrow except to say that it was cellular.

Diffuse interstitial pneumonia was widespread and was characterized by moderate to severe thickening of the alveolar walls, swelling of the alveolar-lining epithelial cells, and accumulation of several macrophages and protein-rich fluid in the alveoli. A search for parasites however was fruitless.

The glomeruli had a thickened Bowman's capsule, thickened glomerular tufts, and a modest mesangial cell proliferation. Adhesions of glomerular tufts to Bowman's capsule were not uncommon. The proximal convoluted tubular epithelium had undergone a hydropic change with accumulation of numerous PAS positive granules. The distal and collecting tubular systems were unaffected. Increased fibrosis and proliferation of lymphocytes with few plasma cells were present in the interstitium (Fig. 8). Evidence of proteinuria characterized by intraluminal accumulation of eosinophilic amorphous material was present in most tubules but not in glomeruli (Fig. 8). L-D bodies were not detected.

The small intestine and striated muscle sections were free of parasitized histiocytes in contrast to articular capsule sections in which they were present.

Electron Microscope Findings

Kupffer cells and macrophages of the cords of Billroth as well as monocytes of the hepatic and splenic sinusoids contained clusters of L-D bodies. Most L-D bodies were free and in intimate contact with the sap of the macrophagic cytoplasm, although some were surrounded to a variable degree by a clear halo (Fig. 9). Only a small number of L-D bodies were seen within membrane-lined cavities.

Irrespective of the type or location of the host cell L-D bodies had similar characteristics (Fig. 10). They were uniform, oval

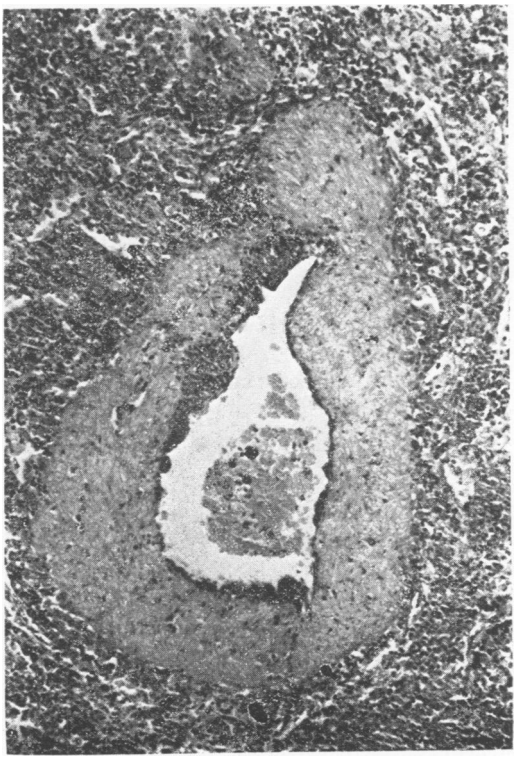

Fig. 6. Splenic vessel. Parasitized cells in endothelial, subendothelial and mural as well as intraluminal location. H & E. X50.

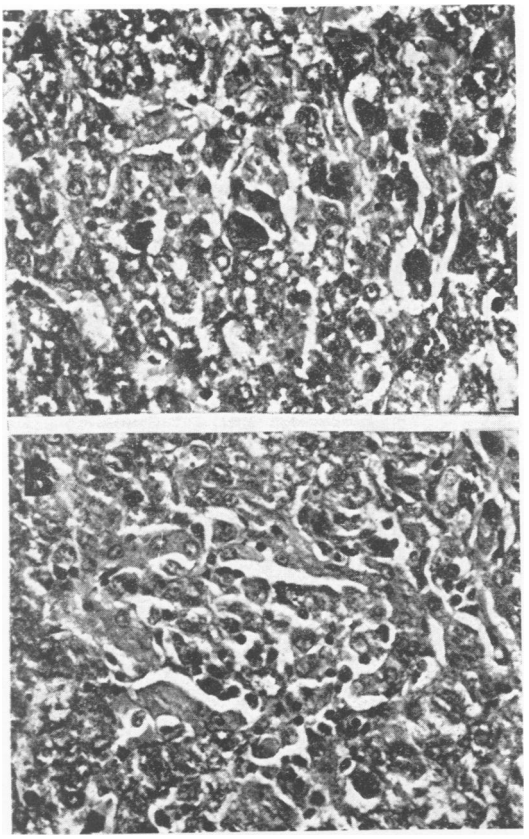

Fig. 7. Liver. A. Enlarged Kupffer cells contain numerous L-D bodies and/or hemosiderin pigment. B. Slight sprinkling of lymphocytes and plasma cells as well as early granuloma formation by parasitized cells. PAS. X345.

structures with mean dimensions of 1.4 x 2.8 μm in median sections, and had a periplast consisting of a double cytoplasmic membrane lined internally by an array of evenly spaced microtubules (Fig. 11a). At the anterior pole of the L-D body and diametrically opposed to the nucleus there was a pocket-like invagination of the periplast harbouring the short flagellum. The pocket and flagellum were lined by two membranes only, the tubular array being absent (Figs. 11c and 11d). The end of the flagellum protruded barely beyond the limits of the anterior pole while its base was continuous with the basal body (Fig. 11d). Cross sections of the flagellum revealed nine peripheral doublets and a central pair of fibrils (Fig. 11b). The fibrils of the central pair were often found to be less well preserved than those of the nine peripheral doublets. The cytoplasm of the L-D contained a variety of organelles some of which were better preserved than others. These included: the Golgi apparatus, a small number of mitochondria, the kinetoplast, a sausage-like structure located near the basal body, lined by two distinct membranes forming *cristae mitochondriales*-like folds, and containing a dense band of twisted or coiled fibrils embedded in a less dense ground substance (Fig. 10, 11c and 11d), an amorphous, moderately electron dense material, presumably lipid and a large number of single or aggregated electron dense granules. The nucleus was round with peripheral dense chromatin and two central nucleoli, one with reduced density (Fig. 11c). The nuclear membrane was distinct and less electron dense than the chromatin.

Parasitized macrophages seemed to be unaffected by the presence of L-D bodies and in spite of the prolonged period of formalin fixation appeared reasonably well preserved.

DISCUSSION

Visceral leishmaniasis was imported into Canada, presumably from Spain, by a dog that had lived in that country for a period of 24 months. Clinical diagnosis was somewhat delayed mainly because the disease is rarely suspected in Canada and was complicated by nephropathy, nonresponsive anemia of unknown etiology, and osteoarthrosis. Thus it proved once more that the iceberg phenomenin, so characteristic of leishmaniasis in its various expressions, is ever present.

Laboratory diagnosis of leishmaniasis is rather easy, certain procedures being more demanding than others. Morphological assessment of bone marrow aspirate is of fundamental value especially when examination of peripheral blood smears does not provide a satisfactory explanation of the underlying etiological mechanisms. In our case it proved to be a decisive factor toward accurate diagnosis. *Leishmania* organisms appear as amastigotes that invade cells of the RES, stain well with Romanowsky type stains, and are easily identifiable by the deeply staining trophonucleus and smaller rod-shaped kinetoplast (30).

Histopathological examination at the light and even electron-microscopic levels is fruitful. Spleen, lymph nodes, liver and

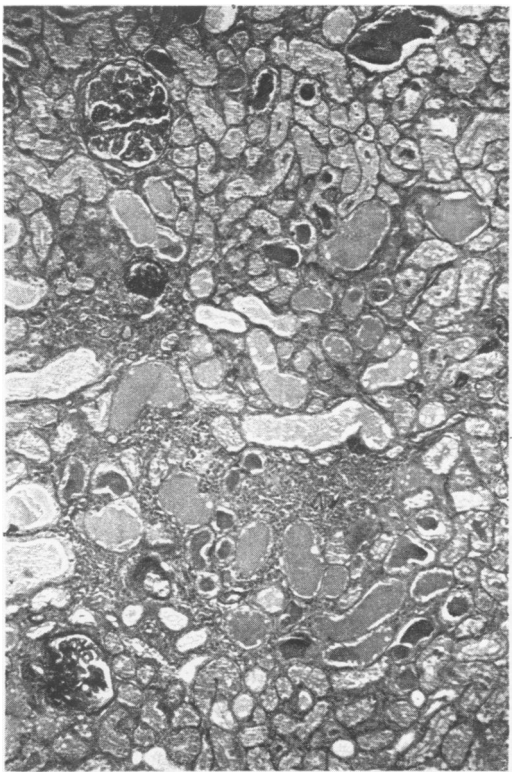

Fig. 8. Renal cortex. Accumulation of lymphocytes and plasma cells in the interstitium, thickening of glomerular basement membranes and hydropic change of epithelial cells with accumulation of proteinaceous fluid in tubules. PAS. X85.

bone marrow under optimal conditions of fixation best lend themselves to such examination. In our case spleen was best suited as it was replete with heavily parasitized RES cells and L-D bodies were easily recognizable in H&E stained sections. Their true density however, was best visualized with the PAMS method.

Electronmicroscopic examination of formalin fixed splenic and hepatic tissues helped to further identify the microorganism and its relationship to the host cell cytoplasm. The leishmanial stage of the parasite was recognized by the presence of: a periplast with two outer membranes and an inner one consisting of an array of evenly spaced tubular fibrils, a nucleus with double nuclear membrane, two nucleoli of different size and density, and prominent marginated blocks of chromatin, a short flagellum in a pocket-like invagination of the periplast, covered by two membranes and containing nine peripheral doublets and a central pair of fibrils, a basal body and a kinetoplast with *cristae mitochondriales*-like folds. The organism can also be recognized by its overall size which ranged slightly around 1.4 x 2.8 μm (2, 24). Although the number of subpellicular tubular fibrils can be a useful criterion of differentiation between *L. donovani* and *L. brasiliensis* a precise morphological differentiation from *L. tropica* is not yet feasible (2, 10, 14, 23).

Biological tests such as complement fixation test, fluorescent antibody test and leishmanin skin test are useful with varying success in the detection of asymptomatic or oligosymptomatic forms as well as to confirm a tentative diagnosis and to identify the leishmanian species involved (19, 20). Biological tests apparently do little to differentiate between strains (26). *In vitro* cultivation of leishmaniae is feasible however and should be attempted whenever possible (20, 26, 29).

In our case the definitive diagnosis of visceral or systemic leishmaniasis caused by *L. donovani* was based partly in retrospect on the history, morphology of the pa-

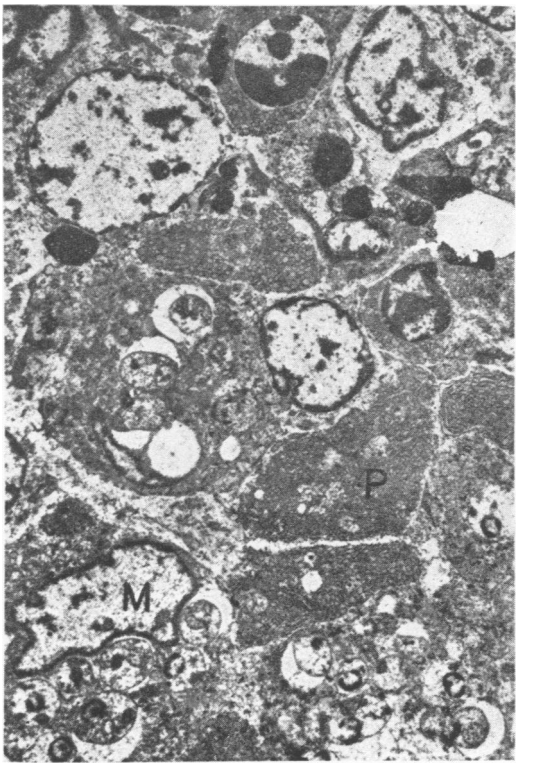

Fig. 9. Splenic red pulp. Aggregates of L-D bodies are visible in the cytoplasm of macrophages (M). A clear halo surrounds partly or completely several L-D bodies. Several plasma cells (P) are recognizable among macrophages. X2355.

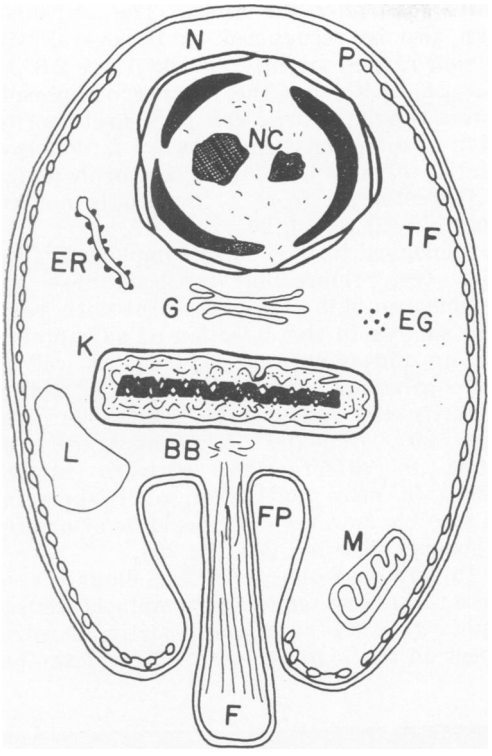

Fig. 10. A diagrammatic synthesis of leishmanial ultrastructural characteristics detected in splenic and hepatic ultrathin sections: BB — basal body, EG — electron dense granules, ER — endoplasmic reticulum, F — flagellum, FP — flagellar pocket, G — Golgi complex, K — kinetoplast, L — lipid, M — mitochondrion, N — nucleus, Nc — nucleoli, P — periplast and TF — tubular fibrils.

rasite in bone marrow smears and tissue sections, and lesions in available organs. The latter included early or advanced granulomatous hepatitis and splenitis with splenomegaly as well as parasitized RES cells in the bone marrow and lymph nodes. Splenomegaly was mainly due to proliferation and enlargement of RES cells many of which formed coalescing granulomata.

Leishmaniasis must be considered in the differential diagnosis of at least the following disorders especially if there is a history of the dog having travelled outside Canada or the U.S.A., namely and in alphabetical order according to author: amyloidosis, canine distemper, glomerulonephritis, intractable dermatopathies, leuko-proliferative diseases, systemic lupus erythematosus, toxoplasmosis, and tuberculosis (27), chronic nonregenerative anemia, hemostatic disorders, generalized lymphadenopathy, hypergammaglobulinemia, intermittent lameness, neoplastic diseases of the hemopoietic organs and ophthalmitis (12), and histoplasmosis (33).

The human and canine counterparts of leishmaniasis have many common characteristics and few dissimilarities. The latter however may be more apparent depending on the stage of the disease or leishmanial strain rather than true (due to species differences). The common features include body weight loss occasionally proceeding to emaciation, intermittent pyrexia, frequent diarrhea, severe nonregenerative anemia, impaired hemostatic function, presence of L-D bodies in the RES cells as well as increased plasma cells in the hematopoietic organs and other tissues, elevated total serum protein associated with reversal of A-G ratio due to polyclonal IgG in man and apparently monoclonal in the dog, nephropathy, splenomegaly and lymphadenomegaly (12, 32).

The most striking differences are limited to the type of blood and bone marrow responses and to a lesser extent in the nature of the hepatic lesion. The peripheral blood leukopenia, granulocytopenia (including eosinopenia), relative lymphocytosis, and frank thrombocytopenia characteristic of the human counterpart of the disease are absent in the dog in which normal WBC counts, relative granulocytosis, normal or slightly depressed lymphocytic counts and inconsistent platelet abnormalities are usually observed (3, 12, 16, 27, 28). In some canine cases, but not in ours, eosinophilic counts were reported to be normal (12, 16). Bone marrow findings typical of the disease in man and not found in the dog are hyperplastic marrow with granulocytic maturation arrest, marked reduction of esinophilic precursors and megakaryocytosis of the non "shedding" type (3) and normoplastic marrow with occasional erythroid hypoplasia and normal granulopoietic activity in the dog. No morphological abnormalities of megakaryocytes were reported. Pancytopenia of human Kala-azar in conjunction with marrow hyperplasia, maturation impairment, and bleeding tendency has been attributed to hypersplenism (3). A similar mechanism may also be operative in canine leishmaniasis since at least one such case with severe hemorrhagic complications has been reported to have improved dramatically following splenectomy (22). Hepatic lesions in man bear similarities to those in the dog except that in man the liver may acquire a cirrhotic character (6, 31).

Finally we share the opinion of others

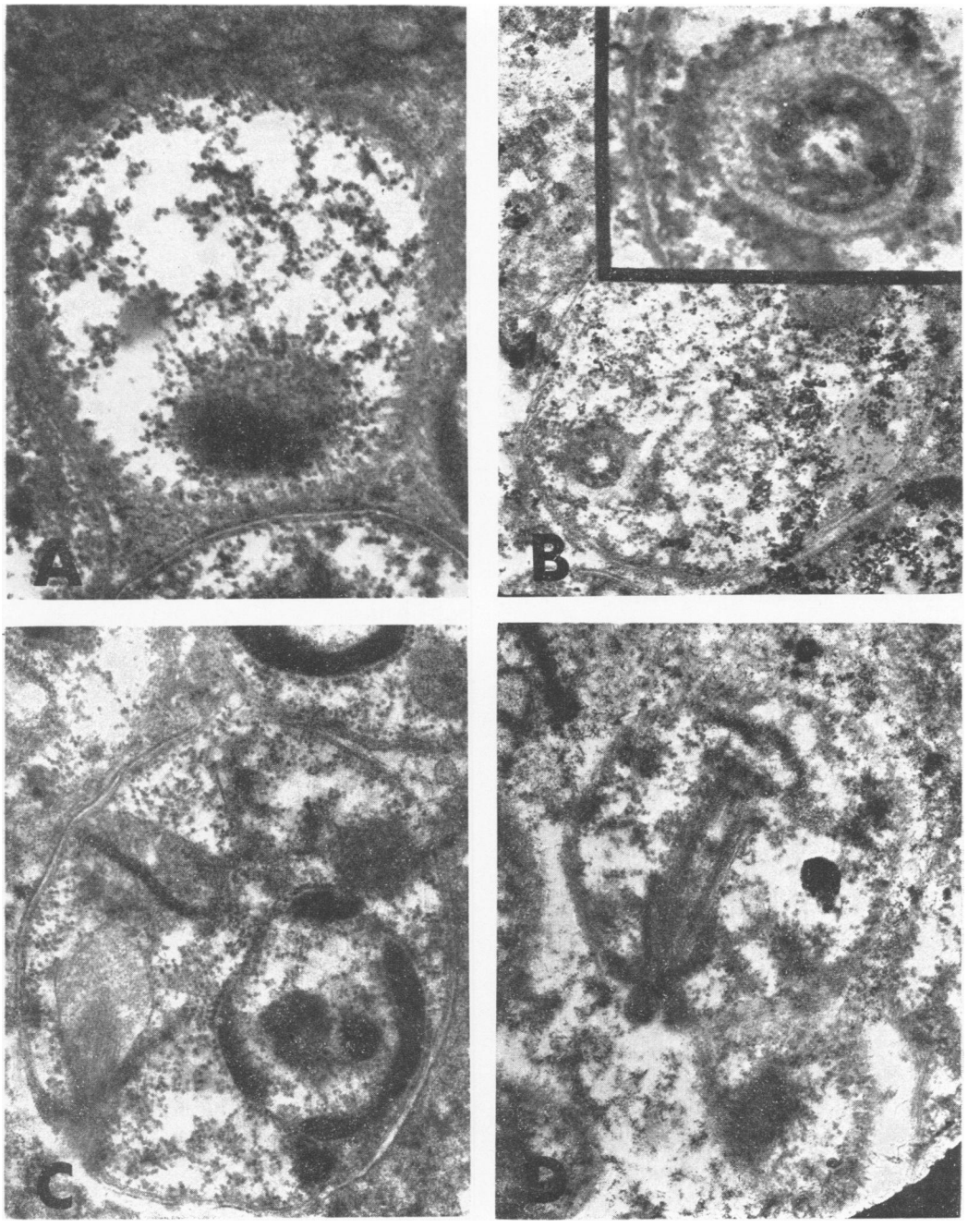

Fig. 11. High power electron micrographs of L-D bodies. A. Periplast consisting of a double membrane and subpellicular tubular fibrils. X67,835. B. Flagellar cross section with pairs of fibrils. Insert: ten distinct pairs are visible. X19,735. C. Flagellar pocket, flagellum, basal body, kinetoplast and nucleus with two nucleoli. X24,665. D. Flagellum, basal body and flagellar pocket. X16,280.

that with increased international travel leishmaniasis may theoretically be a menace to Canada. However, the absence of a reservoir host and the scarcity of vectors render such prospect quite unlikely (8, 12). In view of the possibility, however, of a direct transmission from dog to man as well as from dog to dog great care should be exercised by the examining veterinarian and, needless to say, the owner in protecting themselves or other dogs from becoming infected (12, 27).

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Mrs. H. A. Combley, Mrs. S. M. Fernie and Messrs. I. J. Greer and P. C. Smyth.

REFERENCES

1. **ADLER, S.** Leishmania. Adv. Parasit. **2**: 235-246. 1964.
2. **ANGELOPOULOS, E.** Pellicular microtubules in the family Trypanosomidae. J. Protozool. **17**: 30-51. 1970.
3. **CARTWRIGHT, G. E., H. L. CHUNG and A. CHANG.** Studies on pancytopenia in Kala-azar. Blood **3**: 249-275. 1948.
4. **CONROY, J. D., N. D. LEVINE and E. SMALL.** Visceral leishmaniasis in a fennec fox (**Fennecus zerda**). Pathologia vet. **7**: 163-170. 1970.
5. **COOPER, J. H., B. M. HAG and H. BAGNELL.** Intrafollicular hyalinosis and arterial hyalinosis of the spleen: Histochemical and immunofluorescence studies. J. Path. **98**: 193-199. 1969.
6. **DANESHBOD, K.** Visceral leishmaniasis (**Kala-azar**) in Iran: A pathologic and electron microscopic study. Am. J. clin. Path. **57**: 156-166. 1972.
7. **DAVEY, T. H. and W. CREWE.** A Guide to Human Parasitology for Medical Practitioners. p. 41. London: H. K. Lewis & Co. 1973.
8. **DOWNES, J. A.** Canadian records of **Phlebotomus vexator, Trichomyia nuda,** and **Maruina lanceolata** (diptera:Psychodidae). Can. Ent. **104**: 1135-1136. 1972.
9. **FAUST, E. C., P. F. RUSSELL and R. C. JUNG.** Craig and Faust's Clinical Parasitology. Philadelphia: Lea & Febiger. 1970.
10. **GARDENER, P. J., R. E. HOWELLS and D. H. MOLYNEUX.** Ultrastructure of **Leishmania amastigotes.** Trans. R. Soc. trop. Med. Hyg. **66**: 23. 1972.
11. **GARNHAM, P. C.** American leishmaniasis. Bull. Wld. Hlth Org. **44**: 521-527. 1971.
12. **GILES, R. C., P. K. HILDEBRANDT, R. L. BECKER and C. A. MONTGOMERY.** Visceral **leishmaniasis** in a dog with bilateral endophthalmitis. J. Am. an. hosp. Ass. **11**: 155-159. 1975.
13. **GLEISER, C. A., J. THIEL and I. G. CASHELL.** Visceral leishmaniasis in a dog imported into the United States. Am. J. trop. Med. Hyg. **6**: 227-231. 1957.
14. **HOWELLS, R. E. and P. J. GARDNER.** Preliminary electron microscopic observations on the amastigote forms of **Trypanosoma cruzi** and **Leishmania donovani.** Trans. R. Soc. trop. Med. Hyg. **66**: 336. 1972.
15. **JUBB, K. V. F. and P. C. KENNEDY.** Pathology of Domestic Animals. New York: Academic Press. 1970.
16. **LENNOX, W. J., M. E. SMART and P. B. LITTLE.** Canine leishmaniasis in Canada. Can. vet. J. **13**: 188-190, 1972.
17. **LUNA, L. G.** John's method for kidney. **In** Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. p. 97. New York: McGraw-Hill. 1968.
18. **McCONNELL, E. E., E. F. CHAFFER, I. G. CASHELL and F. M. GARNER.** Visceral leishmaniasis with ocular involvement in a dog. J. Am. vet. med. Ass. **156**: 197-203. 1970.
19. **PAMPIGLIONE, S., P. E. C. MANSON-BAHR, F. GIUNTI, G. GIUNTI, A. PARENTI and G. CANESTRITROTTI.** Studies on Mediterranean leishmaniasis. 2. Asymptomatic cases of visceral leishmaniasis. Trans. R. Soc. trop. Med. Hyg. **68**: 447-453. 1974.
20. **PAMPIGLIONE, S., P. E. C. MANSON-BAHR, M. LAPLACA, M. A. BORGATTI and S. MUSUMECI.** Studies on Mediterranean leishmaniasis. 3. The leishmanin skin test in Kala-azar. Trans. R. Soc. trop. Med. Hyg. **69**: 60-68. 1975.
21. **PEASE, D. E.** Histological Techniques for Electron Microscopy. p. 260. New York: Academic Press. 1964.
22. **RAFYI, A., A. NIAK, R. NAGHSINEH and H. ABOURTORABIAN.** Canine visceral leishmaniasis in Iran. Vet. Rec. **83**: 269-278. 1968.
23. **RONDANELLI, E. G., G. CAROSI, U. CAVALCANTI and G. SPROVIERI.** L'ultrastruttura di Leishmania tropica nel bottone d'oriente. Parasitologia **8**: 333-337. 1971.
24. **RUDZINSKA, M. A., P. A. D'ALESSANDRO and W. TRAGER.** The fine structure of **Leishmania donovani** and the role of the kinetoplast in the **Leishmania-Leptomonad** transformation. J. Protozool. **11**: 166-191. 1964.
25. **SHEFIFF, D.** Canine visceral leishmaniasis in foxhounds near Baghdad. Trans. R. Soc. trop. Med. Hyg. **51**: 467, 1957.
26. **STAUBER, L. A.** Characterization of strains of **Leishmania donovani.** Expl Parasit. **18**: 1-11. 1966.
27. **THERAN, P. and G. J. LING.** Visceral leishmaniasis. J. Am. vet. med. Ass. **150**: 82-88. 1967.
28. **THORSEN, R. E., W. S. BAILEY, B. F. HORLEIN and H. R. SEIBOLD.** A report of a case of imported visceral leishmaniasis of a dog in United States. Am. J. trop. Med. Hyg. **4**: 18-22. 1955.
29. **TOBIE, E. J.** Cultivation of mammalian trypanosomes. J. Protozool. **11**: 418-423. 1964.
30. **UNDRITZ, E.** Sandoz Atlas of Hematology-leishmaniasis. p. 74, plate 249. Basel, Switzerland: Sandoz Ltd. 1952.
31. **VERESS, B., M. O. MALIK and M. A. EL-HASSAN.** Morphological observations on visceral leishmaniasis in the Sudan. Trop. geogr. Med. **26**: 198-203. 1974.
32. **WHITBY, L. E. H. and C. J. C. BRITTON.** Disorders of the Blood. 7th Ed. p. 625. London: J. & A. Churchill Ltd. 1953.
33. **WINSLOW, D. J.** Kala-azar (Visceral leishmaniasis). **In** Marcial-Rojas Pathology of Protozool and Helminthic Diseases. pp. 86-96. Baltimore: Williams & Wilkins. 1970.