FIG. 9: Intraperitoneal inoculation of the lizard *Mabuya striata* with leishmania promastigotes.

DISCARD

FIG. 10: Dissected, Mabuya striata, lizard.

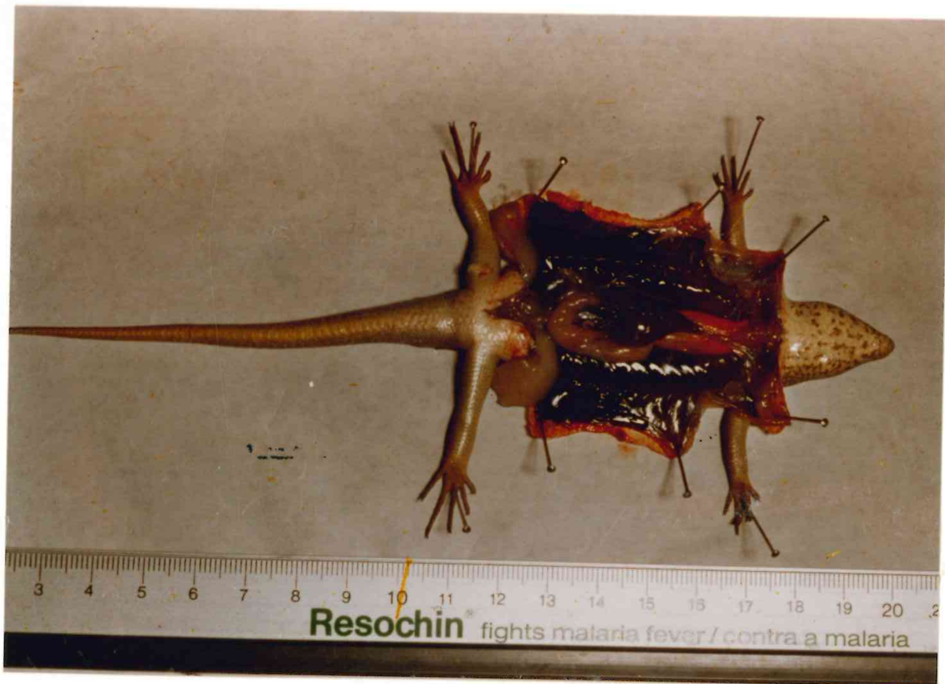

3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Resochin
fights malaria fever / contra a malaria

FIG. 11: Enlargement of the dissected *M. striata*. L. liver; S. spleen; G. gut.

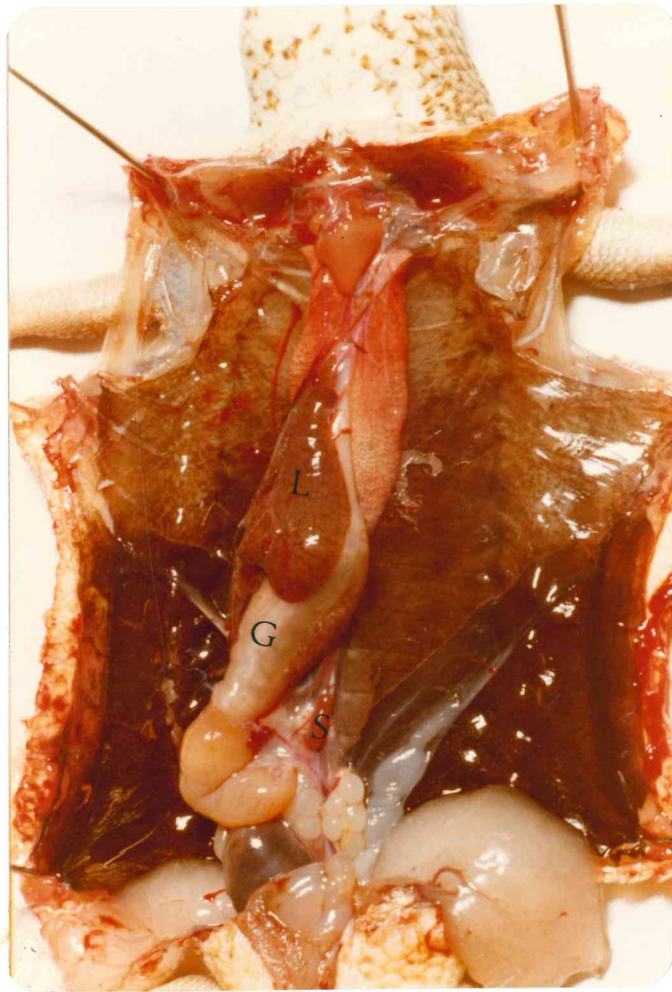

L
G
S

FIG. 12: Dissected white mouse.

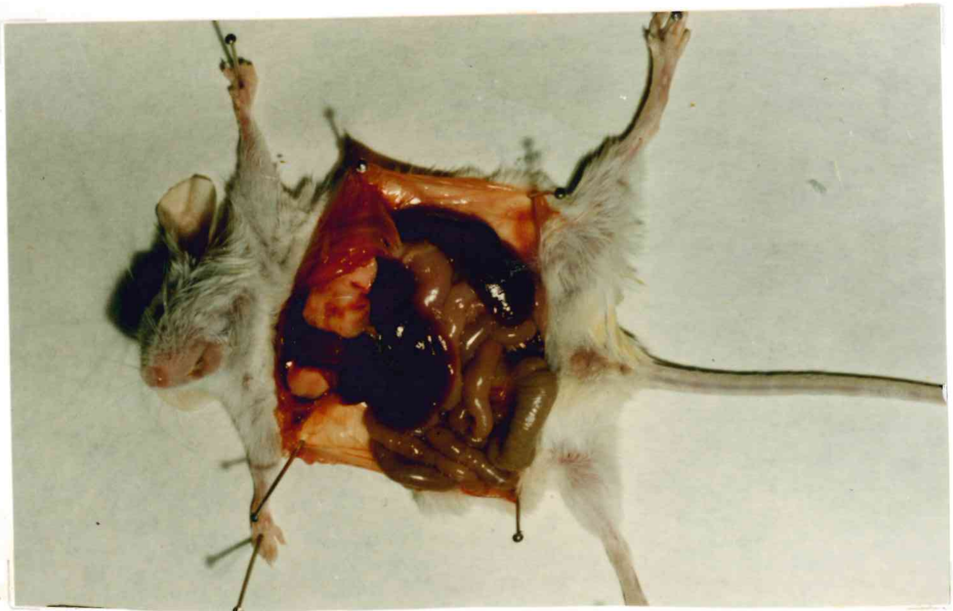


FIG. 13: Enlargement of dissected white mouse to show viscera.
L. liver; S. spleen.

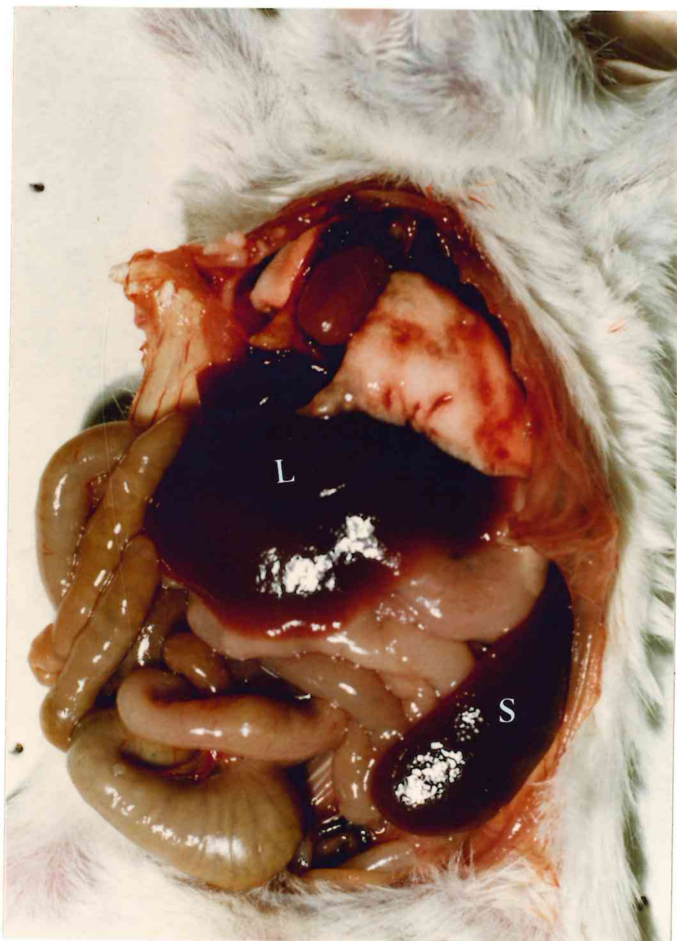
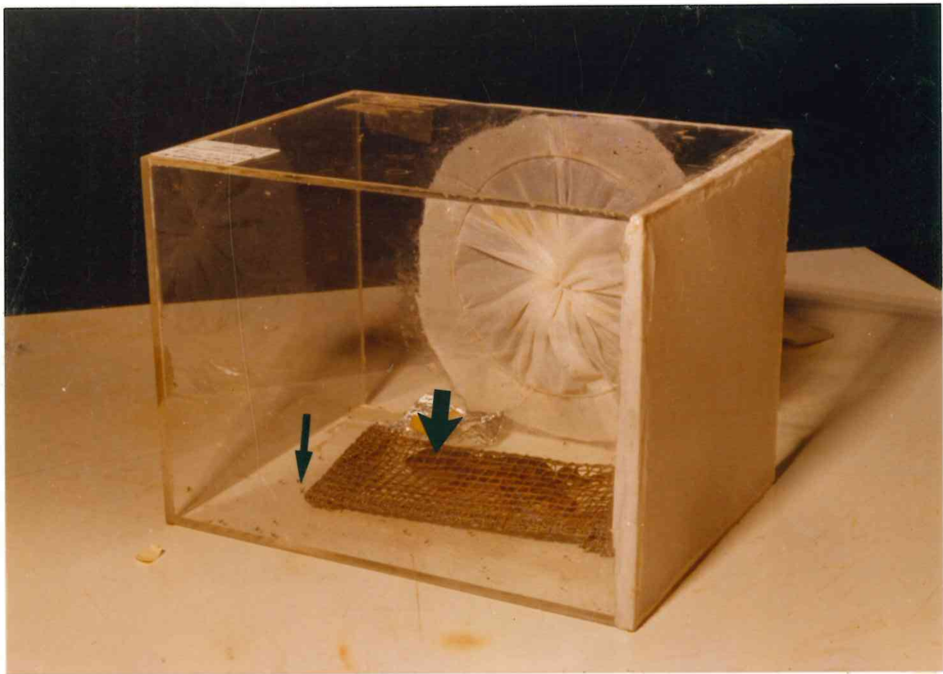

L
S

FIG. 14: Infected lizard (Big arrow) put in a cage with sandflies (Small arrow).



6. Histological Techniques:

6.1. Fixation:

Small pieces of the different tissues needed for histological studies were fixed in 2.5% Glutaraldhyde as stated in 4..

6.2 Paraffin embedding:

The tissues were dehydrated through ascending concentrations of ethanol starting at 50% through 70%, 80% and 90% and two changes of the absolute ethanol. Then they were cleared in toluene overnight and infiltered with paraplast (60°C). The tissues were then taken through three changes of melted paraplast at 60°C in an oven and were blocked in after the third paraffin change.

6.3 Sectioning:

The embedded tissues were serially sectioned at 5 um . Microscopic slides were cleaned in 90% ethanol, dried and smeared with a glycerin-egg-albumin (one part to one part by volume) adhesive mixture. Individual ribbon sections were flattened by heating (45°C) the slide bearing the section floated on distilled water. The slides were left to dry on the slide warmer (Fischer) overnight before staining.

6.4 Staining:

6.4.1 Ehrlich's haematoxylin and eosin (H & E):

The staining essentially involved dewaxing for 10 minutes in 2 changes of xylene dehydrating in descending grades of ethanol and running water for 5 minutes, staining in Ehrlich's haematoxylin for 15 minutes differentiating by immersing in acid alcohol, bluing in running water for 5 minutes, counter staining in 1% eosin for 3 minutes and rinsing in water to remove most of the eosin. The specimens were then dehydrated by ascending grades of ethanol up to absolute, cleared in xylene (2 changes) and mounted using Distrene-Plasticern-xylene (D.P.X.) as a mountant.

7. Electron Microscopy

7.1 Promastigotes

Promastigotes of Leishmania isolates in RPMI-1640 liquid medium (Gibco) were prepared for electron microscopy as follows:

Parasites were spinned at 2,000 r.p.m for 5 minutes. The supernatant was discarded. The parasites were then fixed in 2.5%

Gluteraldehyde in 2.14% sodium cacodylate buffer of 7.4 pH for one hour. The parasites were then spinned at 2,000 r.p.m for 5 minutes before removing the fixative and rinsing them in 2 changes of 2.14% Sodium cacodylate buffer. The parasites were spinned at 2,000 r.p.m. for 5 minutes in each change. Using a fume cupboard the parasites were then post-fixed in 1% Osmium tetraoxide, OSO_4 in Sodium cacodylate buffer for 1 hour then spinned at 2,000 r.p.m. for 5 minutes. The OSO_4 was removed and the parasites were rinsed in 2 changes of 2.14% Sodium Cacodylate buffer. After each change the parasites were spinned at 2,000 r.p.m. for 5 minutes. Then they were block-stained in 50% Uranyl acetate in distilled water.at 4°C for 4 hours. Before removing the stain the parasites were spinned at 2,000 r.p.m. and then dehydrated in grades of Ethanol, 20%, 40%, 60%, 80%, 90% and 2 changes in 100%. They were kept for 5 minutes and spinned at 2,000 r.p.m. in each change. The parasites were then infilterated in 1:1 Spurr's media in Ethanol and kept overnight at room temperature. The infilteration solution was removed after spinning the parasites at 2,000 r.p.m. for 5 minutes and 100% Spur's media was added and left overnight at room temperature. The parasites were then spinned at 2,000 r.p.m. for 5 minutes, and embedded in fresh spurr's media and put in an oven at 60°C for 24 hours.

Semithin and ultrathin sections were cut on LKB ultramicrotomes. The ultrathin sections were placed on pioloform-coated copper grids, and examined with a Zeiss EM 9S2 electron microscope (at the Electron Microscopy Unit, University of Khartoum).

C H A P T E R III

EXPERIMENTS

1. Detection of Developemental Stages of Leishmania sp.in Lizards

1.1 Introduction:

Lizards were proved to harbour Leishmania parasites, but the role they play in spreading human leishmaniasis is not well known. To gain such knowledge it is necessary to follow the developmental stages of Leishmania parasites in experimentally susceptible lizards. Experimental inoculation of lizards using Leishmania isolates of different origin has been done in very few occasions e.g. Mohiudin (1958), Medina (1966, 1968) and Belova (1971).

Leishamania was isolated from wild lizards in Kenya (Ngoka & Mutinga, 1978). One of the isolates, namely LIZ/KEN/75/ICIPE 140 (L140), was used in the present study together with an identified lizard isolate, L. adleri and a human isolate, L. major. The isolate L140 was tested biochemically by Okot-Kotber et al. (1984) who reported it to be biochemically identical to L. major.

1.2 Experimental Design:

This experiment was carried out in two parts. The objective of part 1 was to see the time required for the transformation from promastigotes to amastigotes in lizard tissues. Groups of 2 - 5 *Mabuya striata* lizards were each inoculated with approximately 1 x 10^6 promastigotes of L140 isolate each time. The different groups were dissected at different time intervals, namely 3.5 hours, 7 hours, 14 hours, 3 days and 5 days. Smears of the intraperitoneal fluid (IPF), spleen and liver were made and stained with Giemsa stain and then examined under a light microscope.

In part 2 of this experiment, a total of 180 *Mabuya striata* lizards were used. Lizards were divided into three main groups. Each group formed of 60 lizards which were inoculated with one of the isolates, L140, *L. major* or *L. adleri*. Each lizard was inoculated with approximately 1 x 10^6 - 2 x 10^6 promastigotes. Lizards were sacrificed and dissected at different time intervals, namely 5, 10, 15, 20, 25 and 30 days post-inoculation. From each dissected lizard tissue smears were made from the liver and spleen and were stained with Giemsa stain. Cultures were also made in NNN medium of the heart blood,

liver and spleen. Smears were examined using light microscope. The number of macrophage cells and amastigotes per macrophage cells were counted using a hand tally counter to measure infectivity of the different isolates. Some of the promastigotes cultivated from the cultures were fixed on a slide with absolute methanol and stained with Giemsa.

1.3 Results and Discussion:

Results of part 1 of this experiment are shown in Table 3 in which one can see the occurrence of L140 parasites in various tissues of Mabuya striata lizards. The infection rate among the inoculated lizards was 100% i.e. all the lizards inoculated at different time intervals were parasitaemic. In all lizards dissected at 3.5 hours and 7.0 hours promastigotes were seen in the various tissues, IPF, liver and spleen. Amastigotes were seen in the tissues of all the groups dissected between 7.0 hours and 5 days post-inoculation. These results show that the isolate L140 is infective to the lizard Mabuya striata macrophage cells in vivo and that promastigotes are transformed to amastigotes within 7.0 hours post-inoculation.

Results of part 2 of this experiment are shown in Tables (4), (5) and (6). Table 4 shows the results of inoculation of

M. striata lizards with the isolate L140. There was no significant difference between the counts done in spleen and liver. In both cases amastigotes were seen in macrophage cells of all lizards dissected up to day 20 post-inoculation. No amastigotes were seen in any of the lizards dissected on 25 or 30 days post-inoculation. Day 10 post-inoculation showed the highest percentage of macrophages with amastigotes, 60% and also the highest mean number of amastigotes in macrophage cell, 12.8 ± 6.0. There was a considerable decrease in infectivity of this isolate to the lizards as time increased. The disappearance of amastigotes after day 20 post-inoculation suggests that infection of the isolate L140 to the lizards could be of a transient nature.

Results of inoculation of M. striata lizards with L. major are shown in Table (5). Again here the difference in the liver and spleen counts of amastigotes in the macrophage cells was not significant. Although the percentages of macrophage cells infected are lower than in the case of L140 isolate, all the lizards dissected up to day 20 post-inoculation had amastigotes in their macrophage cells. Eight of the ten lizards inoculated in each case were found infected at day 25 post-inoculation and 7 of 10 lizards at day 30 post-inoculation.

Lizards harbouring amastigotes at 25 and 30 days post-inoculation had relatively low percentage of macrophage cells with amastigote, 4% - 5% and 2% - 3% respectively. Day 10 post-inoculation also had the highest percentage of macrophage cells with amastigotes, 15%, but not the highest mean number of amastigotes in macrophage cell. The highest mean number of amastigotes in macrophage cell was counted on day 5 post-inoculation, 6.2 ± 1.3. The decrease in infectivity between day 5 and day 30 post-inoculation was also noticeable.

Table (6) illustrates the results of inoculation of M. striata lizards with L. adleri promastigotes, there was no significant difference in the liver and spleen counts of amastigotes in macrophage cells. All the lizards dissected between day 5 and 10 post-inoculation had no amastigotes in their macrophage cells. At day 15 post-inoculation amastigotes were seen in 3% of the macrophage cell of 2 lizards with the mean of 0.3 ± 0.7 amastigotes per macrophage cell. The highest number of lizards harbouring amastigotes were 9 out of 10 dissected at day 30 post-inoculation. The percentage of macrophage cells with amastigotes was the highest at day 30 post-inoculation, 25% and also that day had the highest mean number of amastigotes in macrophage cell 2.0 ± 1.1. Hence, it is clear that there is an

increase of infection of lizards between day 5 and day 30 post-inoculation, unlike the former two isolates. Both L140 and L. major had acted differently from L. adleri which is a typical lizard Leishmania. The histogrames in figures (15) and (16) showed clearly these differences both in the percentages of infected macrophage cells and the number of amastigotes per macrophage cell.

Figure (17) shows the promastigotes of the isolate L140 in the intraperitoneal fluid (IPF) 3.5 hours post-inoculation. Figures (18), (19) and (20) show the amastigotes of L140, L. major and L. adleri in the macrophage cells of the lizard tissue. They also show the difference in load of infection between the three isolates, the highest being of L140 followed by L. major then L. adleri as shown by the amastigotes count in the macrophage cells. Amastigotes of Leishmania parasites were seen in lizard tissues in very few occasions, (Shortt & Swaminath, 1928; Rioux et al, 1969; Medina, 1966, 1968). Demonstration of transformation is an indication of a host-parasite relationship and that part of the parasite life cycle is taking place in the vertebrate host, lizard.

Results of heart blood culture of the lizards inoculated with L140, _L. major_ and _L. adleri_ are shown in Table (7). As the table shows, the number of lizards found positive in cultures were lower than the number found positive in smears, i.e. some of the lizards were positive in smears and negative in culture. However, in most such situations negative cultures showed bacterial contamination. Considering the overall picture of the culture results we find that they confirm the same pattern of smears results. The rate of infection decreased between days 5 and 30 post-inoculation in the case of L140 and _L. major_ while it increased between days 5 and 30 post-inoculation in case of _L. adleri_. However, it could be useful to use both smears and cultures to detect infection in lizards.

Table 3: Occurrence of parasites in various tissues of *Mabuya striata* after inoculation with L 140

LIZARD		TIME		RESULTS	
Group	Number	Post-injection	IPF	Spleen	Liver
1	3	3.5 (H)	P	P	P
2	3	7.0 (H)	P	P + A	P + A
3	2	14.0 (H)	A	A	A
4	2	3.0 (D)	A	A	A
5	4	5.0 (D)	A	A	A

KEY:

A = Amastigotes

P = Promastigoets

H = Hours

D = Days

IPF = Intraperitoneal fluid

Table 4: Inoculation of <u>Mabuya striata</u> with L 140 promastigotes

Days Post-inoculation	Number of Lizards		% Mc with A		A/Mc $\bar{X} \pm$ SD	
	Inoculated	Parasitimic	Liver	Spleen	Liver	Spleen
5	10	10	55%	56%	10.5±3.5	10.3±3.9
10	10	10	60%	60%	12.8±6.0	12.7±5.1
15	10	10	40%	35%	3.7±1.2	3.7±1.3
20	10	10	40%	35%	5.3±1.2	5.6±1.2
25	10	0	0	0	0	0
30	10	0	0	0	0	0

<u>KEY</u>:

Mc = Macrophage cells

$\bar{X}$ = Mean number

A = Amastigotes

SD = Standard Deviation

Table 5: Inoculation of *Mabuya striata* with *L. major* promastigotes

Days Post-Inoculation	Number of Lizards		% Mc with A		A/Mc $\bar{X} \pm SD$	
	Inoculated	Parasitimic	Liver	Spleen	Liver	Spleen
5	10	10	10%	8%	5.9±1.1	6.2±1.3
10	10	10	15%	12%	3.2±1.0	3.4±1.3
15	10	10	10%	10%	2.2±1.4	2.2±1.4
20	10	10	9%	8%	2.2±0.9	1.7±0.7
25	10	8	5%	4%	1.1±0.7	1.0±0.0
30	10	7	3%	2%	1.0±0.8	1.0±0.0

KEY

Mc = Macrophage cells   A = Amastigotes

$\bar{X}$ = Mean number   SD = Standard Deviation

Table 6: Inoculation of _Mabuya striata_ with _L. adleri_ promatigotes

Days Post-Inoculation	Number of Lizards		% Mc with A		A/Mc $\bar{X} \pm$ SD	
	Inoculated	Parasitimic	Liver	Spleen	Liver	Spleen
5	10	0	0%	0%	0	0
10	10	0	0%	0%	0	0
15	10	2	3%	3%	0.3 ± 0.7	0.2 ± 0.6
20	10	8	12%	10%	1.3 ± 0.9	1.3 ± 0.9
25	10	8	20%	15%	1.8 ± 1.0	2.0 ± 0.0
30	10	9	25%	15%	2.0 ± 1.1	2.0 ± 1.1

KEY

Mc = Macrophage cells

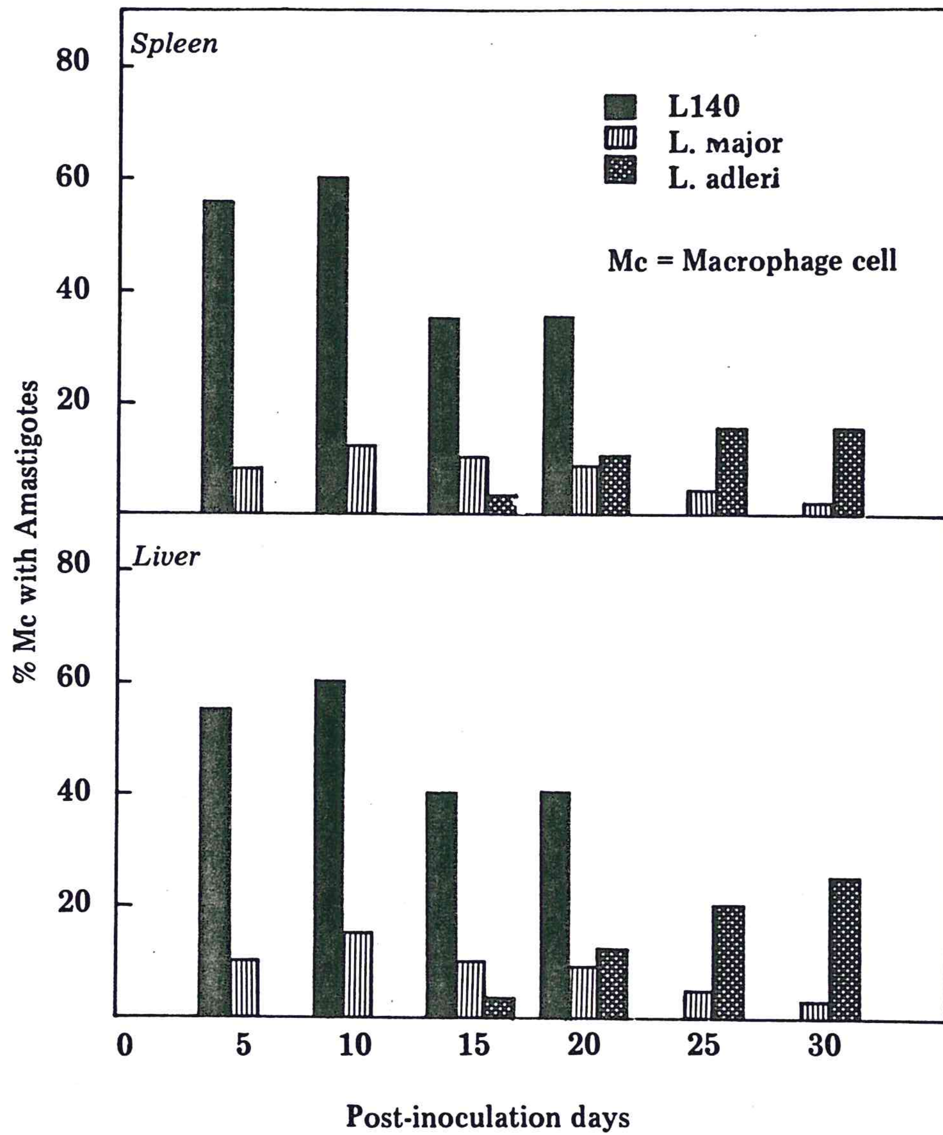
A = Amastigotes

$\bar{X}$ = Mean number

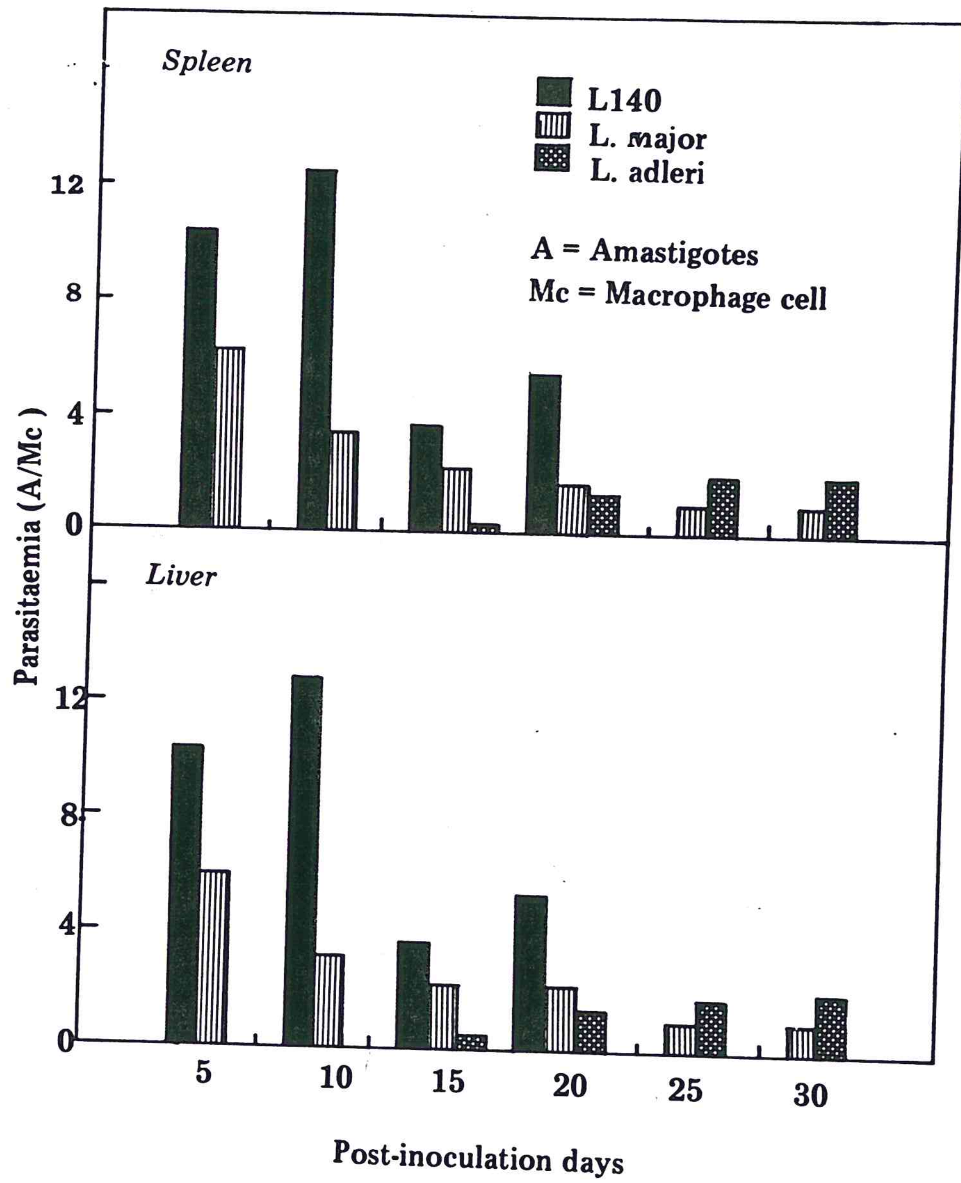
SD = Standard Deviation

Table 7: Results of heart blood cultures of lizards inoculated with L 140, *L. major* and *L. adleri*

Days Post-Inoculation	L 140		*L. major*		*L. adleri*	
	Number of Lizards		Number of Lizards		Number of Lizards	
	Inoculated	Positive	Inoculated	Positive	Inoculated	Positive
5	10	3	10	2	10	0
10	10	2	10	2	10	0
15	10	2	10	1	10	0
20	10	2	10	0	10	2
25	10	0	10	1	10	2
30	10	0	10	1	10	2



Spleen
Liver
L140
L. major
L. adleri
Mc = Macrophage cell
% Mc with Amastigotes
80
60
40
20
80
60
40
20
0
5
10
15
20
25
30
Post-inoculation days



Spleen
L140
L. major
L. adleri
A = Amastigotes
Mc = Macrophage cell
12
8
4
0
Liver
12
8
4
0
Parasitaemia (A/Mc)
5
10
15
20
25
30
Post-inoculation days

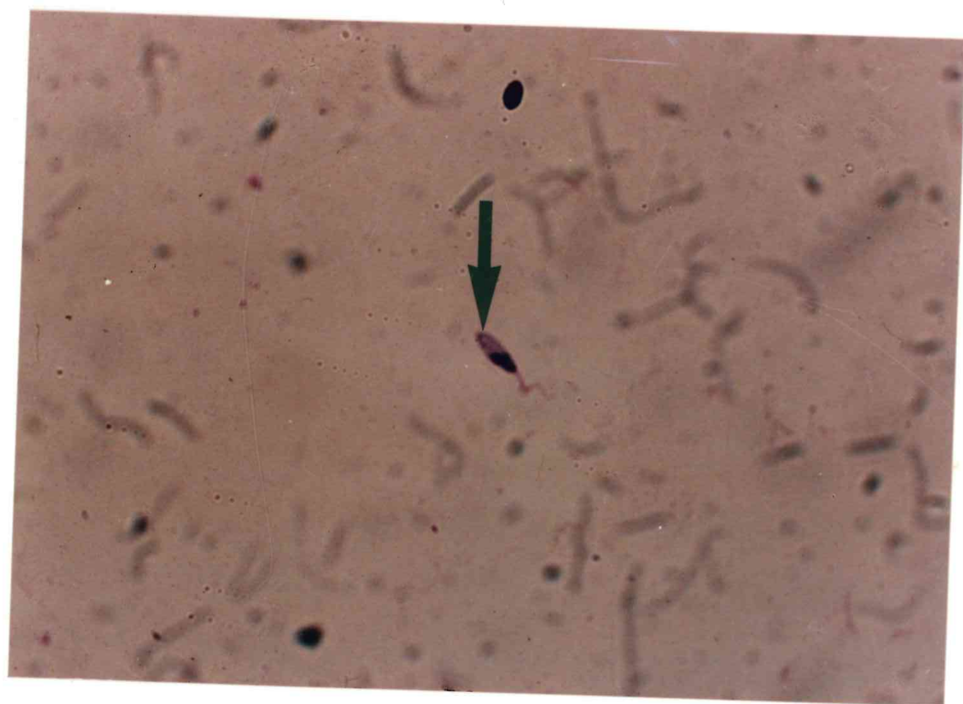


FIG. 18: Impression smear of a lizard liver infected with L 140. A, amastigotes; N. nuclei of ruptured macrophage cells; R, red blood cell. Magnification 325 X.

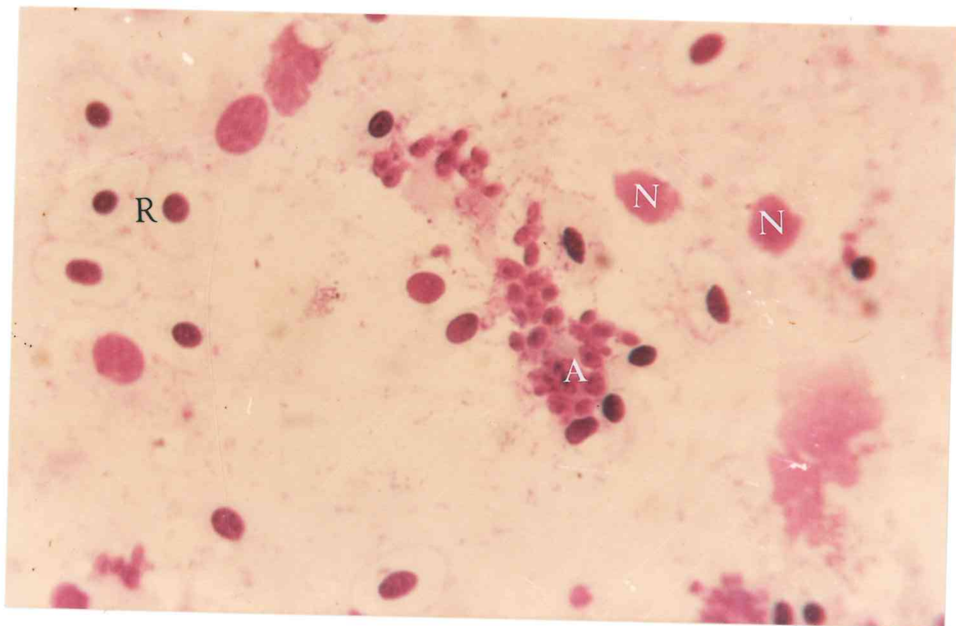

R
N
N
A

FIG. 19: Impression smear of a lizard liver infected with L. major. A, amastogotes; N, nuclei of ruptured macrophage cells; R, red blood cell. Magnification 325 X.

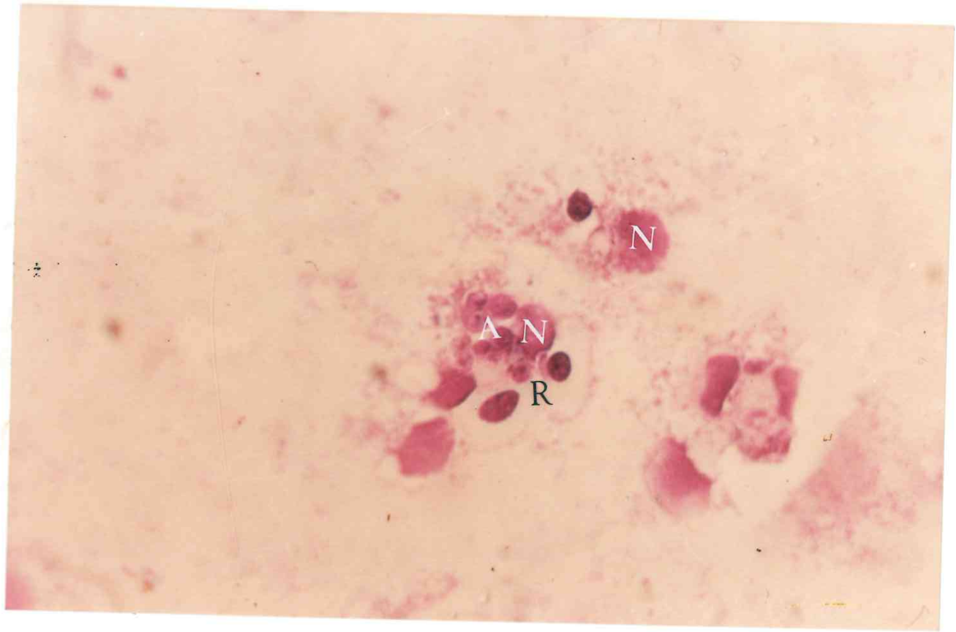
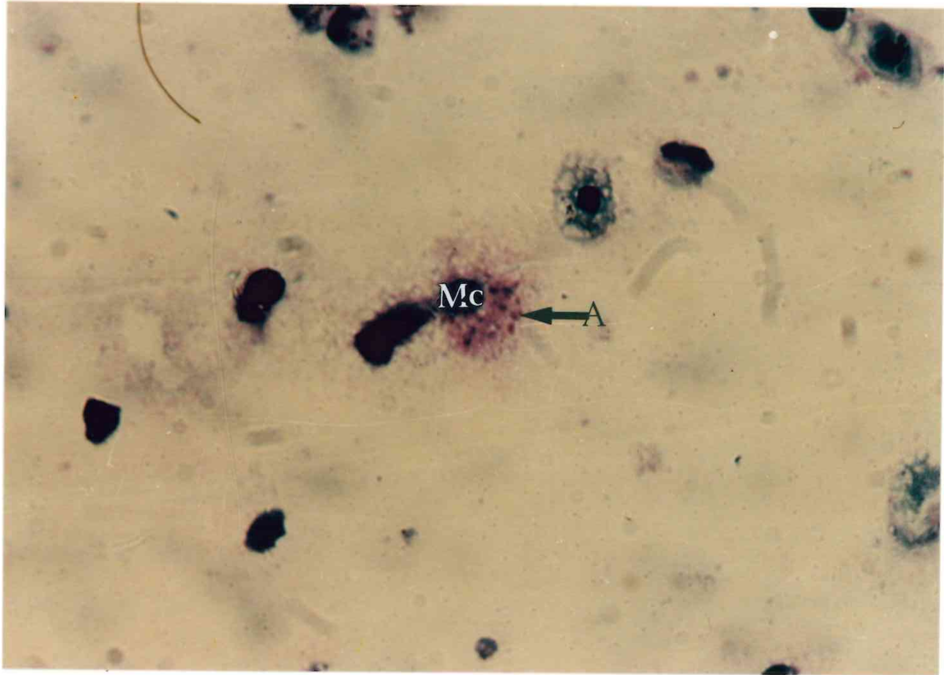

N
A
N
R

FIG. 20: Impression smear of a lizard liver infected with *L. adleri*
A, amastigote; Mc, macrophage cell.
Magnification 325 X.


Mc
A

2. Experimental Infection of Mice with Lizard *Leishmania* isolates

2.1 Introduction:

Animals infected with *Leishmania* may or may not show obvious signs of infection. The long term co-evolution of parasite and natural host tends to result in none or a few harmful effects to the host (WHO, 1984).

In this experiment mice were experimentally infected with *L. adleri* and L140 parasites to test the infectivity of the lizard isolate, L140, to mice and compare it with *L. adleri* infectivity.

2.2 Experimental Design:

A total number of 220 mice were used in this experiment. One group of 80 mice were infected intraperitoneally with *L. adleri* promastigotes. Each mouse was inoculated with $5 \times 10^6 - 10^7$ promastigotes. The second group consisted of 120 mice which were inoculated with L140 promastigotes. Half of this group, 60 were inoculated intraperitoneally (IP). The other half were injected half the dose in the base of the tail and the other half in the tip of the nose (NT). The full inoculum given to

each mouse was 5 x 10^6 - 10^7 promastigotes. Twenty mice were kept as controls for the two groups.

Ten mice of each group and two of the controls were dissected weekly starting from the inoculation day up to the 8th week in the case of the group injected with L. adleri and to the 12th week in the case of the group injected with L140. Smears of the spleen and liver were done, stained with Giemsa and examined under X 100 lense of a light microscope. Four smears were examined from each organ for detection of amastigotes.

2.3 Results and Discussion:

Results of this experiment are shown in tables 8 and 9.

Table (8) shows the results of inoculating mice with L. adleri promastigotes. No amastigotes were seen in all the mice dissected in the first week post-inoculation. In the second week 3 mice showed amastigotes both in the liver and spleen macrophage cells. Four of the mice harboured amastigotes in the third week and 3 in the fourth week post-inoculation. All the mice dissected from the fifth week to the eighth week post-inoculation showed no amastigotes in all the smears examined. The number of

amastigotes seen per macrophage cell was between 1 and 3. Six of the inoculated mice died between the second and the fourth week post-inoculation and were all found to harbour parasites. These results confirm the results obtained by Adler (1962) who demonstrated cryptic and transient infections in mice inoculated with L. adleri up to five weeks after inoculation.

Table (9) shows the results of inoculating mice with L140 promastigotes. All the mice dissected between week 1 to week 8 post-inoculation were found to be free of parasites. The percentage of mice found to harbour parasites between week 9 and 12 post-inoculation ranged from 40% to 80% in both (NT) and (IP) groups. Post-mortem carried out on the dead mice showed that all the mice which died between week 9 and 12 post-inoculation harboured parasites. Figures (21) and (22) show amastigotes of L 140 and L. adleri in mice macrophage cells.

Considering infectivity of both isolates, L. adleri and L140, we find that while L. adleri had transient infectivity L140 seemed to have more persisting infectivity to the mice. This could mean that L140 isolate showed characteristics with mammalian parasites. Young & Hertig (1927) produced visceral,

followed by cutaneous lesions in the Chinese hamster *Cricetus griseus* after inoculating them with *L. tarentolae*. Hence, there is also a possibility that L140 isolate was from reptilian origin, but the fact that it was found biochemically identical to *L. major* (Okot-Kotber *et al.*, 1985) gives more support to the suggestion that it may be from a mammalian origin or it possesses shared characteristics with both reptiles and mammals.

Table 8: Inoculation of Mice with L. adleri promastigotes

Weeks Post-Inoculation	Number of Mice Inoculated	Number of Mice with amastigotes	Number of Mice Dead	% of Mice with amastigotes
1	10	0	0	0%
2	10	3	1	30%
3	10	4	3	40%
4	10	3	2	30%
5	10	0	0	0%
6	10	0	0	0%
7	10	0	0	0%
8	10	0	0	0%

Table 9: Inoculation of Mice with L140 promastigotes

Weeks Post-Inoculation	Number of Mice							
	N T				I P			
	Inoculated	With amastigotes	Dead	%	Inoculated	With amastigotes	Dead	%
1	5	0	0	0	5	0	0	0
2	5	0	0	0	5	0	0	0
3	5	0	0	0	5	0	0	0
4	5	0	0	0	5	0	0	0
5	5	0	0	0	5	0	0	0
6	5	0	0	0	5	0	0	0
7	5	0	0	0	5	0	0	0
8	5	0	2	0	5	0	0	0
9	5	3	2	60	5	4	2	80
10	5	4	2	80	5	4	3	80
11	5	2	1	40	5	4	1	80
12	5	3	2	60	5	4	1	80

KEY:

NT = Group of mice inoculated in the nose and tail

IP = Group of mice inoculated intraperitoneally.

FIG. 21: Impression smear of a mouse liver infected with L 140.
A, amastigotes; N, nucleus of macrophage cell.
Maginification 325 X.

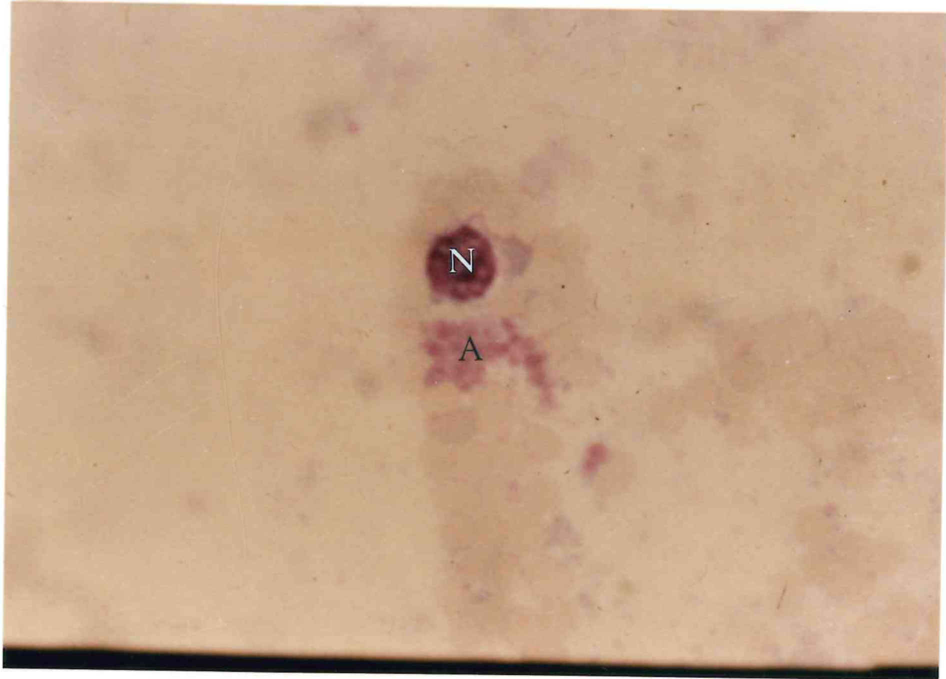
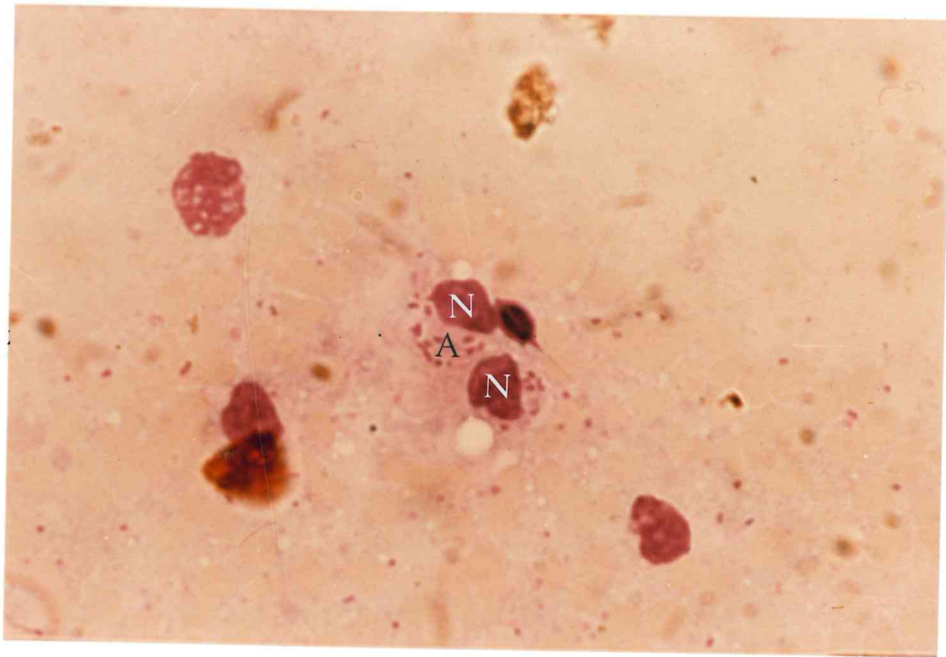

N
A

FIG. 22: Impression smear of a mouse liver infected with *L. adleri*.
A, amastigotes; N, nuclei of macrophage cells.
Magnification 325 X.


N
A
N

3. Experimental Feeding of Sandflies on Infected Lizards

3.1 Introduction;

All forms of human leishmaniasis are known to be transmitted by the bite of the female phlebotomine sandfly. Nevertheless, the actual mechanism of transmission is still somewhat obscure. Sandflies of many species have been shown experimentally to be susceptible to infection with almost any species of Leishmania. Many attempts to study transmission have been made, with few successes and many failures, the reasons for which are not understood (Johnson & Hertig, 1970).

Sandflies of the species Sergentomyia bedfordi, S. rodhiani, S. schwetzi, S. antennatus, S. affinis and S. africanus feed mainly on reptiles and occasionally they have been observed to bite man (Mutinga & Ngoka, 1981). Some of these species and other species were selected to be used in this experiment.

The aim of this experiment was to determine the infection rate of sandflies after feeding on infected lizards and also to locate the position of parasites, if seen, in the sandfly gut.

3.2 Experimental Design:

Four species of laboratory-bred sandflies were used in this experiment. Different numbers of the four species were used: 190 of S. ingrami, 130 of S. schwetzi, 20 of S. clydei and 15 of S. bedfordi. One hundred and fifty-five flies were fed on lizards inoculated with the isolate L140, 120 flies fed on lizards inoculated with L. adleri and 80 flies fed on lizards inoculated with L. major. In the case of L140 and L. major, lizards were put in fly cages between day 5-10 post-inoculation while in the case of L. adleri lizards were put between day 25-30 post-inoculation.

The fed flies were dissected between day 1 and 15 post-feeding and the gut was examined for parasites under a light microscope.

3.3 Results and Discussion:

Table (10) shows the result of feeding sandflies on infected lizards. Figures (23) and (24) show sandflies feeding on the lizards. Of the 155 sandflies fed on lizards inoculated with the isolate L140 and dissected, none revealed developing

parasites in the gut. The same was found true for the 80 flies which fed on lizards inoculated with L. major. As for the 120 flies which fed on lizards inoculated with L. adleri 2 sandflies were found to harbour promastigotes. These were S. schwetzi and S. ingrami. In both cases flies were dissected at day 6 post-feeding, and promastigotes were seen in the anterior midgut of the infected flies. The number of promastigotes seen were few and the gut was free of bacteria. Bacteria was seen in 90% of the other dissected sandflies. Presence of bacteria in the sandfly gut inhibit the development of parasites, (Chung et al., 1951; Adler, 1964). This could be the reason for the low infection rate among the fed flies (0.6%). Infections of the sandflies were the result of ingesting relatively few amastigotes with the infecting blood meal from the lizards. This explains why the number of promastigotes seen were few (Killick-Kendrick, 1979). Promastigotes seen in both flies have adopted anterior position in the sandflies gut. The anterior position has been accepted as a criterion for human Leishmania infections while lizard Leishmania species were known to adopt a posterior position. The results obtained here confirm that some lizard Leishmania species can adopt an anterior position which is an indication that transmission to the lizard can be by the bite of an infected fly.

Table 10: Feeding sandflies on infected lizards

Isolate Inoculated into Lizards	FLIES: Species (*Sergentomyia*)	Number: Fed & Dissected	Number: With Parasites	% Infected
L 140	*schwetzi*	50	0	0
"	*ingrami*	70	0	0
"	*bedfordi*	15	0	0
"	*clydei*	20	0	0
L. major	*ingrami*	80	0	0
L. adleri	*schwetzi*	80	1*	1.3
"	*ingrami*	40	1*	2.5
Total number		355	2	0.6

All flies were dissected between the days 1 - 15 post-feeding.

* Both infected flies were dissected on day 6 post-feeding.

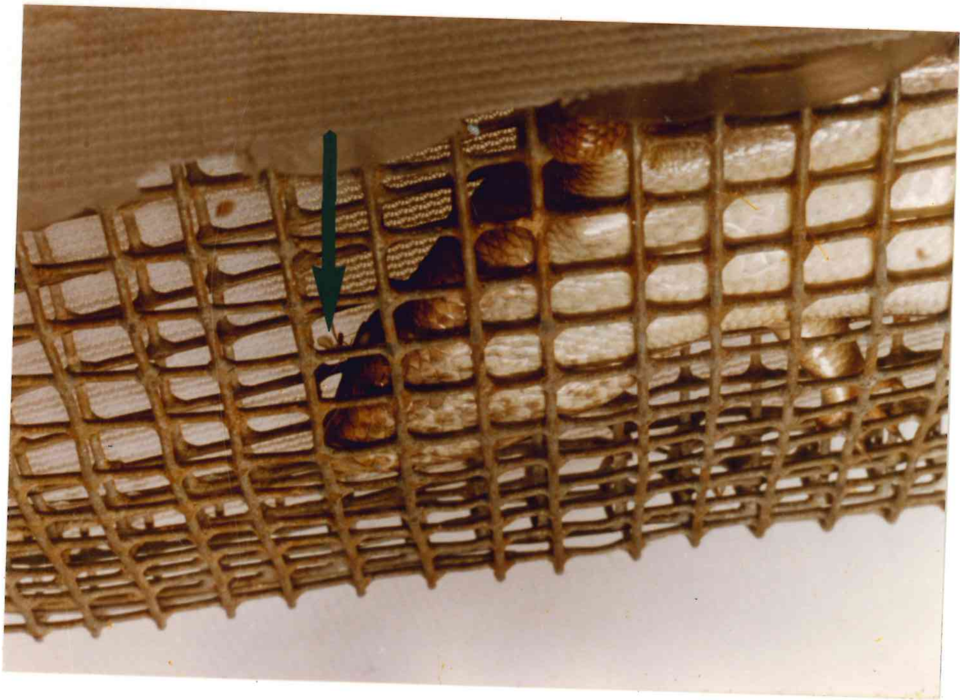


FIG. 24: Engorged sandflies still feeding on an infected lizard.

4. Histological and Ultrastructural Observations

4.1 Leishmania in Histological Sections of Lizard Tissues

The sections made of the infected lizard tissues were 5 u thick and were stained in H. & E. Leishmaniae amastigotes of both L 140 and L. major were detected in the macrophage cells of the liver, spleen and gut of the infected lizards.

It is not known whether Leishmania parasites have any effect on lizards. No change in behaviour or activity of the infected lizards was observed during the course of this study. Hence a histopathological study through [illegible] sections will be useful to know how lizard tissues and cells react to infection.

4.2 Promastigotes Ultrastructure of a Leishmania (L 140) Isolate

The investigations of the fine structure of promastigote forms of Leishmania from reptiles are few. These include Trager & Rudzinska (1964), who reported the riboflavin requirement and the effects of acriflavin on the fine structure of kinetoplast of L. tranentolae. Lewis (1975) had described the ultrastructure of some reptilian Leishmaniae compared with human Leishmania.

Kaddu & Mutinga (1981) studied the fine structure of L. aethiopica promastigotes isolated from the midgut of naturally infected P. pedifer. Given below is a description of the basic fine structure of promastigotes as observed in the present study:

Pellicle and Associated Organells - The surface of Leishmania promastigote appears to lack any coat. One can sometimes observe a very thin diffuse layer adhering to the external surface of the cell membrane, (Figures 30-32). Beneath the cell membrane are the longitudinally arranged subpellicular microtubules, (Figures 30, 33). Spacing of the subpellicular microtubules was measured and found to range between 26.6-28.0 nm. which is more related to the mammalian strains as reported by Garnham (1971) and Lewis (1975).

Flagellum and Associated Organelles - the axoneme consists of the usual 9 pairs of peripheral tubules surrounding a central pair (Figures 30, 31). The whole extracellular flagellum is bounded by a trilaminate flagellar membrane, (Figure 31). The axoneme continues to a point near the base of the flagellar pocket (Figures 31, 33). The flagellar pocket expands and forms a reservoir (Figures 31, 33).

Mitochondrial System and Kinetoplast - The kinetoplast lies near the base of the axoneme tubules (Figures 31-33). The mitochondrial system is a branched structure originating from the kinetoplast, (Figures 30, 33).

Cytoplasmic Systems - Cytoplasmic inclusions are seen in some of the sections (Figures 31, 32). Endoplasmic reticulum was observed around the nucleus and runs parallel to the pellicle, (Figure 30). According to Lewis (1975), the endoplasmic reticulum is well developed in mammalian <u>Leishmania</u> than in reptilian.

FIG. 25: Histological section of a lizard liver infected with L 140. Amastigotes (arrows) in macrophage cells. Magnification is 325 X.

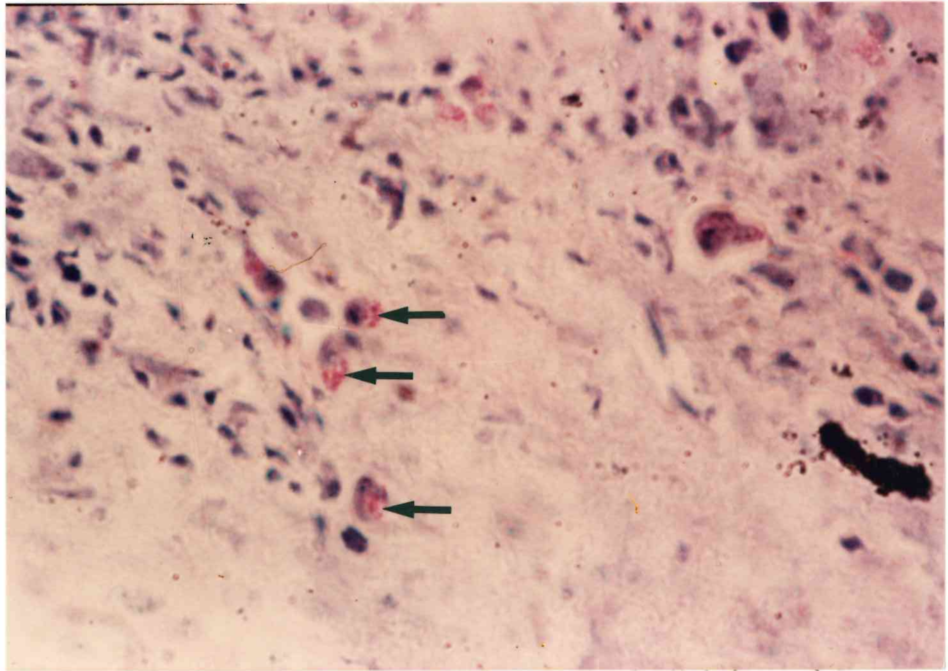


FIG. 26: Histological section of a lizard spleen infected with L 140. Amastigotes (arrows) seen in the cell. Magnification 325X.

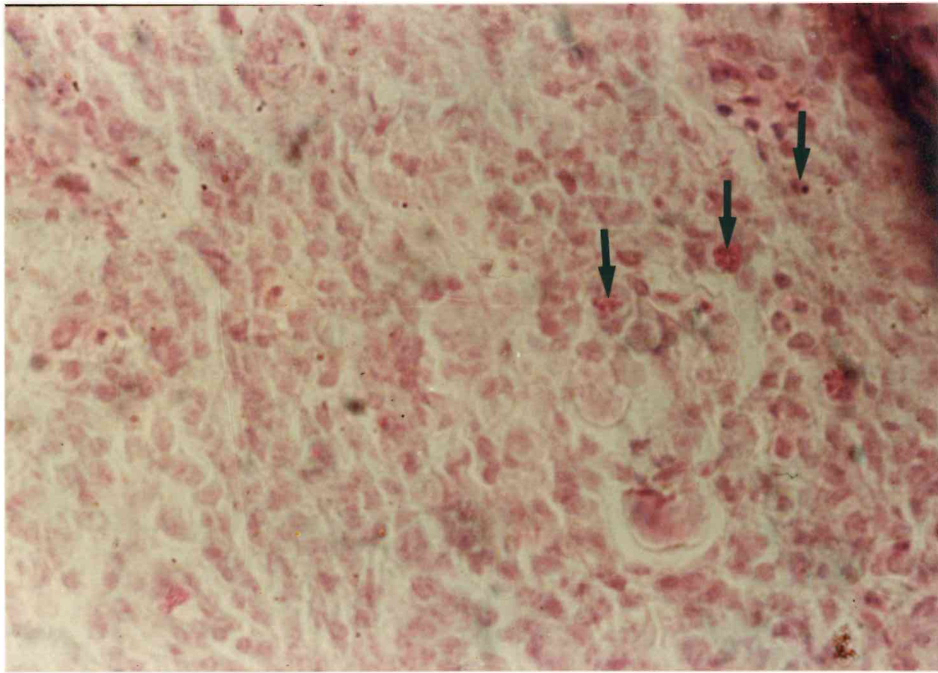


FIG. 27: Histological section of a lizard gut infected with L 140. Amastigotes (arrow) in macrophage cell. Magification 235X.

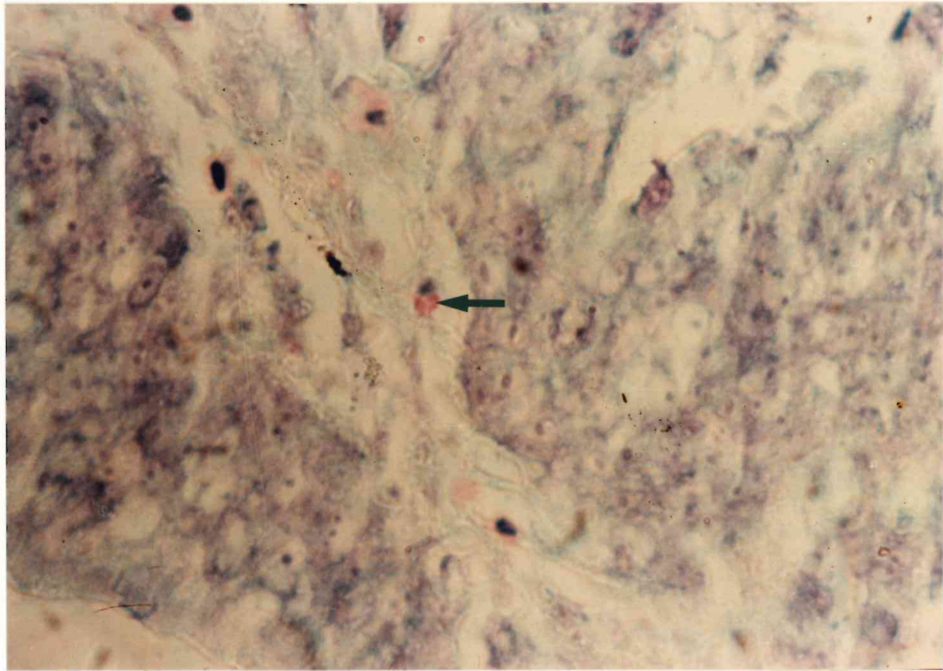


FIG. 28: Histological section of a lizard liver infected with *L. major*. Amastigotes are shown with arrows. Magnification 325X.

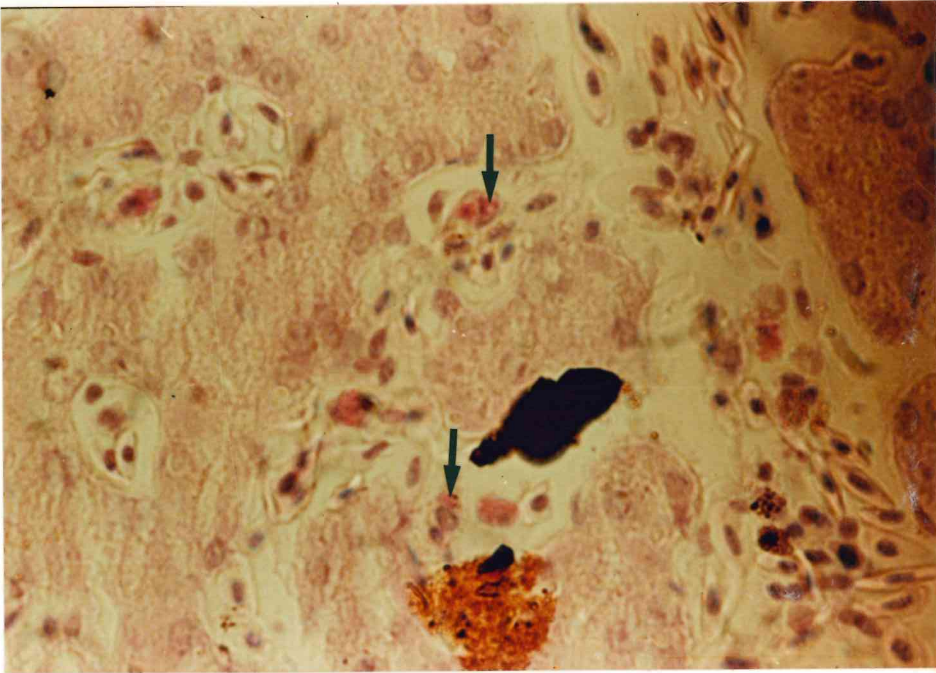


FIG. 29: Histological section of a lizard spleen infected with *L. major*. Amastigotes in the cells are shown with arrows. Magnification 325X.

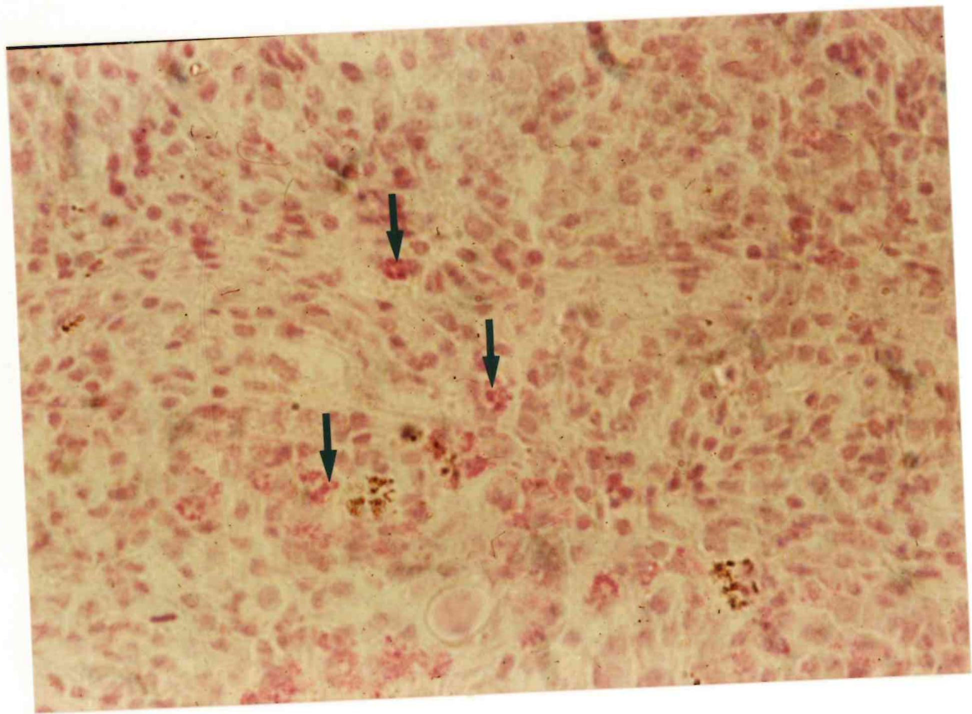


FIG. 30: Electron micrograph of oblique section of L 140 promastigote. Cm, trilaminate cell membrane; S, subpellicular microtubules; Er, endoplasmic reticulum; M, mitochondria; F, flagellum; N, nucleus; Ne, nucleolus. Magnification 45,000X.

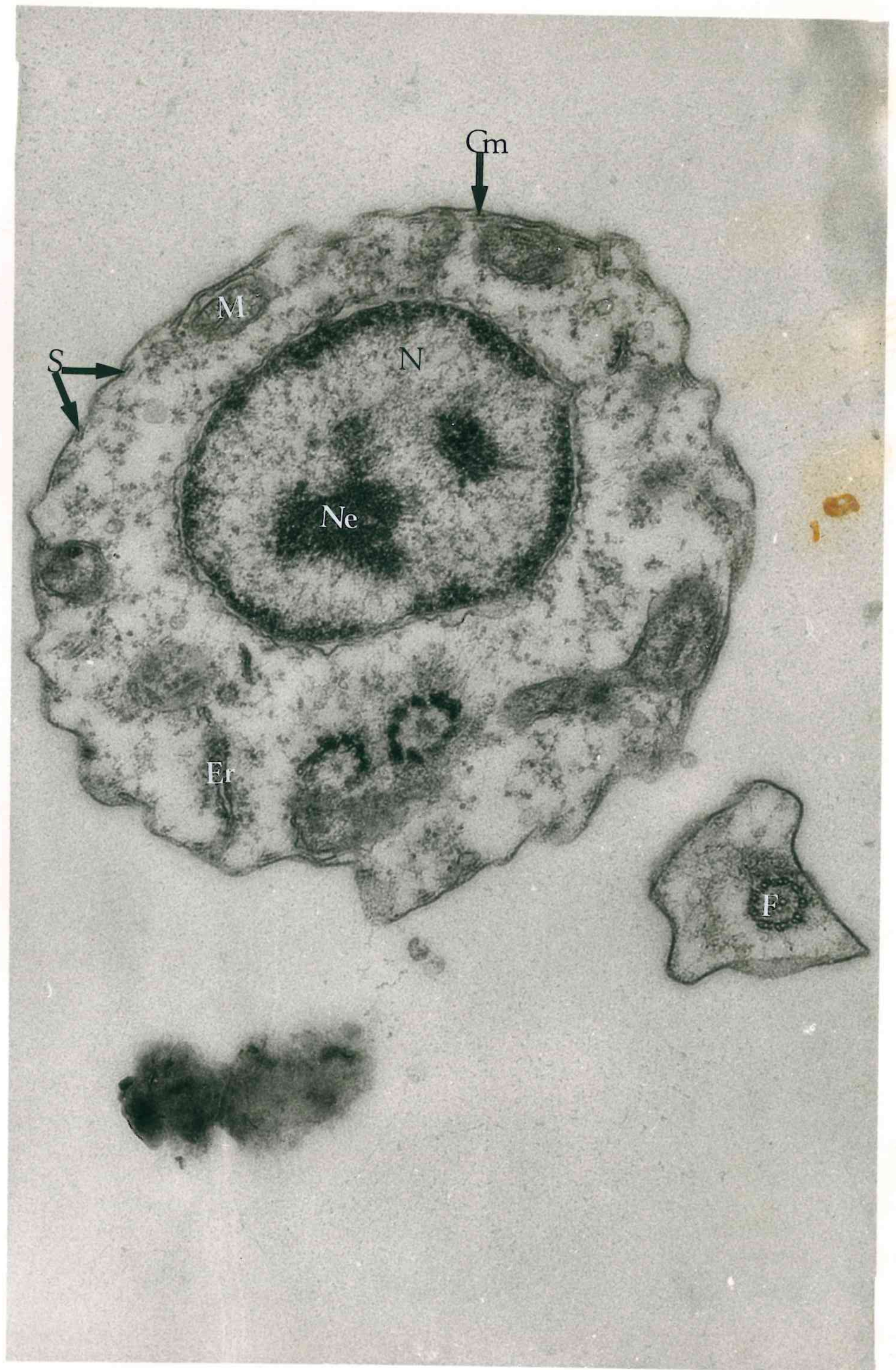

Cm
M
S
N
Ne
Er
F

FIG. 31: Electron micrograph of longitudinal section of L 140 promastogote. K, kinetoplast; M, mitochondria; R, flagellar pocket reservoir; A, axoneme, N, nucleus; Ne, nucleolus; Cm, cell membrane; F flagellum; I, cytoplasmic inclusion; V, vacuole. Magnification 32,500X.

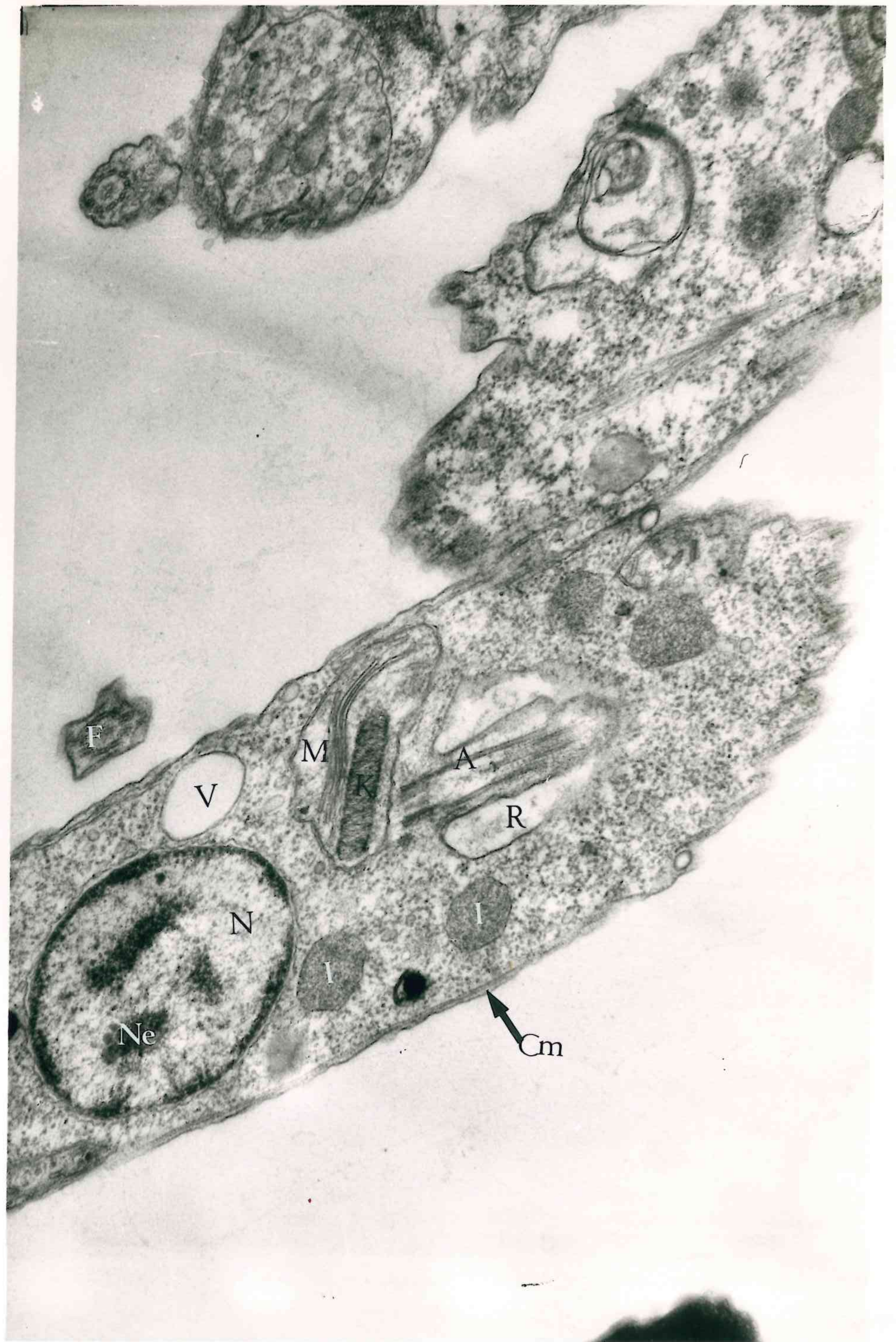

F
M
K
A
V
R
N
I
I
Ne
Cm

FIG. 32: Enlarged electron micrograph of a section through the kinetoplast of L 140 promastigote. K, kinetoplast; N, nucleus; Ne, nuclear envolope F, flagellum. Magnification 97,500X.

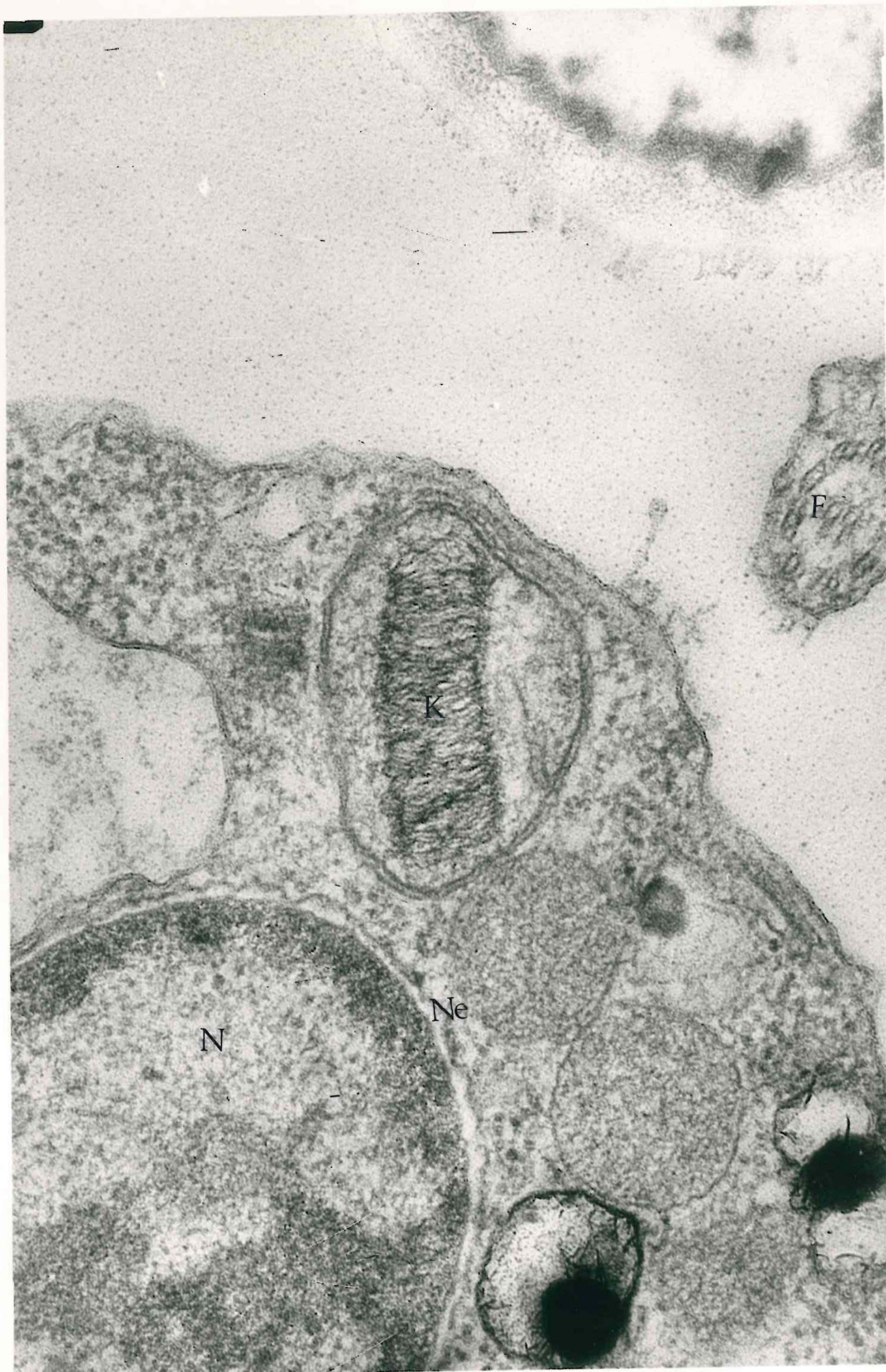
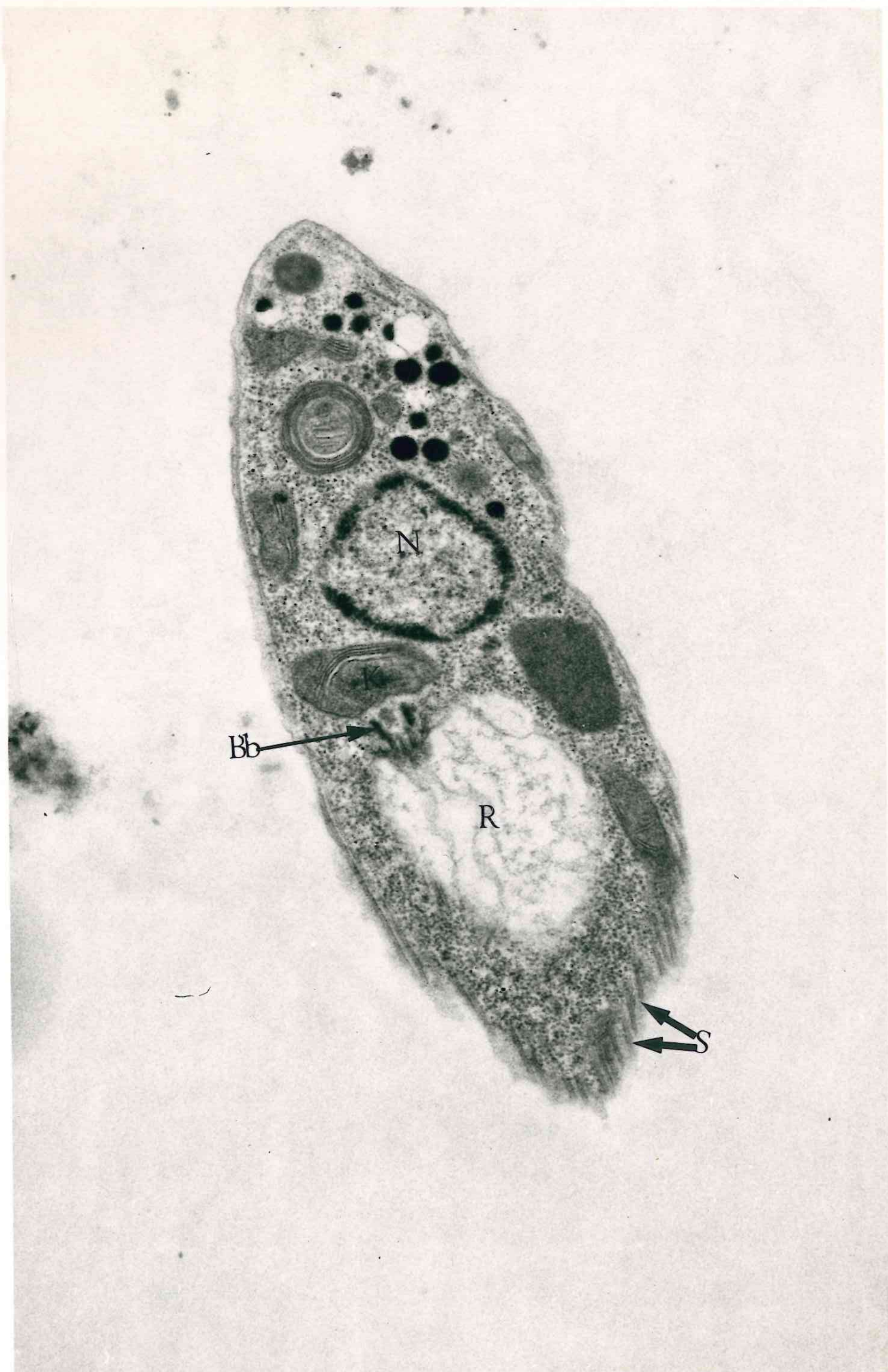

F
K
Ne
N

FIG. 33: Electron micrograph of L 140 promastigote showing a transverse section through the kinetoplast. K, kinetoplast; N, nucleus; Bb, basal body; R, flagellar pocket (reservoir); S, subpellicular microtubules. Magnification 50,000X.

I



C H A P T E R IV

GENERAL DISCUSSION

It has been shown in the present study that transformation of *Leishmania* promastigotes to amastigotes had taken place in the lizard tissues following an itraperitoneal inoculation with *Leishmaniae* promastigotes. There have been very few experimental infections where promastigotes and amastigotes were seen in smears of the lizard organs (Dollahon & Janovy, 1974; Belova, 1966; Medina, 1966, 1968). In this study both promastigotes and amastigotes of three *Leishmania* isolates, namely L140, *L. major* and *L. adleri*, were seen in the lizards tissues. It was stated by Adler (1964) that transformation of promastigotes to amastigotes can be the way of finding a solution to the problem of transmission.

Infectivity of the isolate L140 was tested both in lizards and mice and the results obtained showed that this isolate is more likely to be of a mammalian origin. Its infectivity to the lizards is of a transient nature while it was more persisting in mice. The results were more comparable to *L. major* than to *L. adleri*. If lizards could harbour human leishmaniae, they can then act as reservoir hosts or incidental hosts and play an important role in epidemiology of human leishmaniasis (Belova, 1971). *Leishmania* were isolated from

girbles in Baringo District by Mutinga & Ngoka (1978). Their isolate has been typed together with the strain isolate by Heich *et al*. (1959) from the same area and were both found to be *L. major* (Chance *et al*., 1978). *L. major* was again reported to be isolated from rodents in Baringo district of Kenya (Githure & Beach, 1984). *Leishmania* (L140) was isolated from lizards in Kacheliba, West Pokot to the N. West of Marigat, Baringo (Ngoka & Mutinga, 1978). This is within the same area where *P. duboscqui*, which is the vector for *L. major* has been reported (Beach *et al*, 1982). Hence, supported by the experimental results obtained in the present study, it is possible that lizards can act potentially as reservoir hosts for *L. major* being in the same habitat with the mammalian hosts and the vectors. It is believed that transmission of lizard *Leishmaniae* to human population can confer a degree of immunity and prevent infection with human *Leishmania* (Latyshev & Lryukova, 1953; Van Peenen & Deithein, 1963; McMillan, 1965; Southgate & Manson-Bahr, 1967; Mutinga & Ngoka, 1983). Some sandfly species such as *S. bedford*, *S. rodhiani*, *S. schwetzi*, *S. antennatus*, *S. affinis* and *S. africanus* can feed both on reptiles and mammals (Mutinga & Ngoka, 1981) and carry infection from infected lizards as shown in the present study. Thus an integral epidemiological studies on leishmania sis should include lizard *Leishmaniae*. Ingram &

Molyneux (1983, a, b; 1984, a, b) have carried out a series of immunological studies on Agama causpinosum and Lacerta viridis using L. agamae cultures. Their results provide a guide for further studies on the immunological role lizards can play in leishmaniasis. This role could be in the heterologous immunity hypothesis stated by Cahill (1970) as being very important in the control of visceral leishmaniasis. His hypothesis was that immunity can be induced by vaccination with avirulent leishmaniae.

Experimental infection of sandflies by feeding on infected animals can lead to the mode of transmission and to the natural vectors. Most experiments have been carried out by feeding sandflies on cultured promastigotes (Kaddu & Mutinga, 1986), more work need to be carried out using amastigotes to compare the behaviour of the parasites. The life cycle of any saurian leishmania is not yet fully known. There is doubt about the suspected vectors reported for five of the known eleven lizard leishmania (Table 1) (Killick-Kendrick, 1979). Leishmania tarentolae is the best studied saurian parasite, but observations on its life cycle in the fly are conflicting (Parrot, 1934 a, b; 1935; Adler, 1933). Sergentomyia clydei was reported by Heisch (1958) to be the vector of L. adleri. None of the 20 S. clydei

used in the present study was infected after feeding on lizards inoculated with *L. adleri*. Heisch (1954) also reported *S. clydei* to feed on gerbils from which he (1958) had isolated a strain of *Leishmania* different from *L. adleri*. Results of experimental studies on the life cycle of *L. agamae* in *P. papatasi* were reported by Adler & Theodor (1957). Feeding of *P. papatasi* on cultures of *L. agamae* promastigotes resulted in heavy infection at the posterior station of the flies. No promastigotes were found in the sandflies guts when they were fed on infected lizards. The authors concluded that transmission of *L. agamae* is not by bite but through ingestion, and that *P. papatasi* was not the natural vector. Development of *L. ceramodactyli* in *P. papatasi* was also studied by Adler & Theodor (1929a) who showed that the promastigotes developed in the posterior station of the fly with anterior spread only when infections were intense. The ease with which *P. papatasi* fed on geckos, the high susceptibility of this sandfly and the speed of development of the parasites led to the suggestion that *P. papatasi* might be the natural vector of *L. ceramodactyli* and that transmission must be by the oral route. *Leishmania gymnodactyli* of lizards in the USSR develops only in the mid and hind guts of both the experimental host, *P. papatasi*, and the natural vector, *S. murgabiensis*. Transmission of the species seems to be by ingestion (Saf'janova & Alekseen, 1967).

The life cycle of *L. hoogstraali* is not known. Experiments carried out by McMillan (1965) showed no development of the parasites in *P. papatasi* and Laison *et al* (1977) got only one scanty infection in *Lu. longipalpis*.

Development of parasites in the sandfly gut can be affected by many factors. Some of the factors could be due to intrinsic insusceptibility of the sandflies. For example, a batch of a species of sandflies taking potentially infective blood meals do not all become infected and, in those that do, infections are not all of the same intensity. It was assumed that there are genetically controlled differences in susceptibility among individuals of the same population (Killick-Kendrick, 1979). It has also been reported that, in the old world, the ecology of the parsite, and in particular the species of fly transmitting it in a given place, moulds the parasite in such a way that its characteristics become different from closely related parasites in other ecological situations (Kirk, 1949). Another factor that can affect the development of parasites in the sandfly is the number of parasites ingested. Sandflies which have low susceptibility to a given strain of *Leishmania* do not normally become infected when they take up only few parasites, but this natural resistance can be overcome if

they ingest artificially high numbers, (Killick-Kendrick, 1979). Highly susceptible sandflies show flourishing infections of the midgut though very few amastigotes may have been ingested (Shortt, 1945; Strangways-Dixon & Lainson, 1966). Time, temperature and humidity were also found to have effect on development of *Leishmania* in the fly. Leaney (1977) found that too high temperature can interfere with the establishment of infection in the midgut and may also inhibit anterior migration to the pharynx and mouthparts. Different species of leishmanias have varied speed of development. *Leishmania mexicana* takes 4 days to develop from taking infecting blood meal to infective bite (Strangways-Dixon & Lainson, 1966; Williams, 1966; Ward *et al*., 1977). *Leishmania infantum* completes its development in 6 days (Adler & Theodor, 1931). In some other species of *Leishmania*, e.g. *L. donovani*, in *P. argentipes*, transmission cannot take place at the first blood meal after the infecting meal (Shortt, 1928). Rioux *et al*. (1978) have reported that development of *L. infantum* in *P. ariasi*, in natural conditions, is not complete until more than 14 days after the infecting blood meal, have passed. Mutinga & Odhiambo (1985) reported that the optimal time for infection of *P. pedifer* is between 9-13 days.

Sandflies feed on sugars (Lewis & Domney, 1966; Chaniots, 1974; Young _et al._, 1978). The frequency with which they are taken and, most importantly, their origin are unknown. Yet it has been suggested that natural sugars may provide an essential substrate for leishmanias in the barren environment of the foregut of the sandfly (Sherlock & Sherlock, 1961; Killick-kendrick et al., 1977). _Leishmania_ development can also be affected by contaminant infections in the sandfly gut which is known to harbour a wide variety of organisms (Young & Lewis, 1977). The gut of adult sandflies is normally sterile. Bacterial or fungal infections usually inhibit the development of _Leishmania_ and kill the fly (Chung _et al._, 1951; Adler, 1964). Among protozoa, the commonest parasites of sandflies are Acephaline eugregarines (Shortt & Swaminath, 1927; Adler & Mayrink, 1961). Sandflies are also known to be vectors of a variety of protozoa of reptiles and amphibia, including trypanosomes, haemogregrines and malaria parasites (Young & Lewis, 1977). However, since this parasitized flies are species which never or only rarely feed on mammals, the parasites are of no direct importance in the transmission of leishmaniasis to man. It is not known if they interfere with the development of saurian leishmanias. The rate of experimentally infected sandflies in the present study was low, (0.6%), although all the flies had fed on the infected lizards. It is suggested that bacterial contamination of the sandflies gut and the

number of parasites taken with the blood meal during feeding of the sandflies are the main factors responsible for such low infection rate.

From what has been discussed, one could conclude that there could be a relationship between reptilian and mammalian leishmaniasis and that, that relationsip could be responsible for most of the irregularity of human infection in leishmania endemic areas where infected lizards are found. The results of the present study suggest that the isolate L 140 could be of a mammalian origin and it could be *L. major*. Since the behaviour of L 140 in mice was found to be different from that of *L. major*, one could put a hypothesis that mammalian *Leishmaniae* change their behaviour when passed through lizards. This hypothesis can be the base for further studies on that line. Hence it is recommended that more work should be done on investigating the identity of leishmaniae isolated from lizards using biochemical characterization. Also it is important to study whether the change in infectivity of the isolates has any significance in inducing immunity in mammals and man to *Leishmania*. In this regard histological and fine structural techniques could be applied together with immunological studies.

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