EXPERIMENTAL TRANSMISSION OF *PLASMODIUM MEXICANUM* BY BITES OF INFECTED *LUTZOMYIA VEXATOR* (DIPTERA: PSYCHODIDAE)[1]

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ABSTRACT. *Lutzomyia vexator* is an efficient experimental vector of *Plasmodium mexicanum*, infecting 69.2% (9/13) of the *Sceloporus undulatus* lizards with as few as one bite. Sporozoites were present in the salivary glands by day 6.5 postfeed and infective by day 8 postfeed at 27°C. The prepatent period was relatively long, ranging from 23 to 40 days for bite-induced infections and appears to be related to the number of sporozoites injected. The acute phase of the infection is initially exponential and rapid. All lizards (6) that were not sacrificed, died of fulminating infections from 13 to 56 days after parasites were seen in the blood films. Gametocytes from 2 experimentally infected lizards were infective to *L. vexator* during the course of the acute infection. The majority of *P. mexicanum* parasites were in erythrocytes of *Sc. undulatus*. Exoerythrocytic forms were observed in circulating lymphocytes and thrombocytes, lymphocytes of spleen and bone marrow, and endothelial cells of brain capillaries.

INTRODUCTION

Saurian malaria research has received increasing attention (primarily taxonomic and ecological) in the past few years, but the natural vectors remain unknown. Fifty-nine species of saurian *Plasmodium* have been described (36, Americas; 11, Australia, Asia and Oceania; 12, Africa), three of which occur north of Mexico (Telford 1982, 1983, 1984a, 1984b; Garnham and Telford 1984). Ayala and Lee (1970), Ayala (1971) and Petit et al. (1983) are the only authors to describe any part of the extrinsic cycle of lizard malaria, *Plasmodium mexicanum* Thompson and Huff and *Plasmodium agamae* (Wenyon), respectively; developing beyond the early oocyst stage. Ayala and Lee (1970) demonstrated that *P. mexicanum* developed in phlebotomine sand flies while Petit et al. (1983) showed that *P. agamae* developed in *Culicoides nubeculosus* (Meigen), and not in mosquitoes as previously suspected. Ayala (1971) further demonstrated sporogony and experimental transmission of *P. mexicanum* by intraperitoneal inoculation of sporozoites from wild caught *Lutzomyia vexator* (Coquillett) females that had earlier fed on infected lizards *Sceloporus occidentalis* Baird and Girard. Although Ayala did not demonstrate transmission of malaria by the bite of infected flies, he did suggest that the natural route of infection is by bite because: (1) sporozoites migrate to the salivary glands, (2) blood feeding lasted a relatively long time, and (3) sporozoites were shown to be infective when injected intraperitoneally in lizards. However, Ayala did not rule out the possibility of transmission by ingestion of infected flies. Transmission of *P. agamae* is also believed to be transmitted by bite but the sporozoites were retained in the oocyst and were not observed in the salivary glands of *C. nubeculosus*, an unnatural host (Petit et al. 1983).

New developments in rearing phlebotomine sand flies (Endris et al. 1982) provided the opportunity for experimental transmission studies of *P. mexicanum*. This study describes the first successful experimental transmission of a malaria parasite by bite of a hematophagous insect other than mosquitoes. The incubation period and course of acute infection of *P. mexicanum* in *Sceloporus undulatus* Latreille, transmitted by bite of *L. vexator*, are also reported.

MATERIALS AND METHODS

The colony of *Lutzomyia vexator*, originated from wild caught females from Gulf Hammock, Levy Co., Florida, in 1981, and was maintained by methods similar to those described by Endris et al. (1982) and Young et al. (1981). Approximately 200 larvae from individual females, reared in 25 ml plastic oviposition vials (12–20 days posteclosion), were transferred to 120 ml urine specimen containers and provided with horn fly medium, which decreased larval development time (Greer and Butler 1973). Adults were released daily from the 120 ml containers into a modified glass aquarium (34 × 21 × 27 cm) and were provided slices of apple as a food source (Endris et al. 1982). All developmental

[1] The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (P. 4-3, AR 360-5). Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals, and adheres to principles stated in the *Guide for the care and use of laboratory animals*, NIH Publication 85-23. Florida Agric. Exp. Station Journal Series No. 7924.

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stages of *L. vexator*, including blood-fed females were maintained in a Hotpack® incubator (temperature, 27 ± 1°C or 24 ± 1°C; relative humidity, 80 ± 5%; and 16:8 LD photoperiod).

Sceloporus occidentalis (western fence lizard) were collected by hand at Rumsey Canyon, 4 km north of Rumsey, Yolo County, California, and examined for the circulating stages of *Plasmodium mexicanum*. Infected lizards were sent to the University of Florida for transmission studies. *Sceloporus undulatus undulatus* (eastern fence lizard), collected from Austin Cary Forest, Alachua County, Florida, were similarly examined for the presence of *Plasmodium floridense* parasites. Thin blood-films were made from a clipped toe, air dried, fixed with absolute methanol, then stained with Giemsa. Subsequent blood-films were prepared by clipping the tail tip. *Sceloporus undulatus* that did not show patent *P. floridense* infections in at least 3 blood-films within a 30 day period were used in *P. mexicanum* transmission studies. Malaria parasites were identified by the authors. Lizards were maintained in screened cages (50 × 25 × 25 cm) in the laboratory at room temperature and provided an external heat source from a 40 watt incandescent light bulb. Lizards were fed house flies (*Musca domestica* L.) and Lepidoptera larvae (*Galleria* sp. and *Spodoptera* sp.). Water was provided ad lib. in petri dishes and by spraying the cages daily.

Laboratory reared *L. vexator* females were fed on *Sc. occidentalis* which demonstrated >1% of the red blood cells (RBCs) infected with *P. mexicanum* gametocytes. Blood-fed sand flies were removed at 4 hr intervals, placed in 25 ml oviposition vials, and provided a sugar source (1:1 mixture of Karo® syrup and distilled water). Midguts were dissected by the method of Chaniotis and Anderson (1968), at intervals from 2 to 7 days postfeed (PF) and examined for oocysts. Beginning on day 5 PF, the salivary glands were examined also, and the sporozoite rate estimated (+1, 1–10; +2, 11–100; +3, >100 sporozoites).

One to six female *L. vexator* infected with *P. mexicanum* sporozoites were placed in a Plexiglas® cage lined with plaster of paris (Endris et al. 1982) and provided a second blood meal on a non-infected, wild-caught *Sc. undulatus*. Lizards fed upon by one or more infected sand flies were placed in a screened cage and maintained as previously described or were placed in a temperature-humidity controlled chamber and maintained at 27°C and 80% RH. Blood-fed female flies were dissected after the second blood meal and the sporozoite rate determined. To determine if transmission of *P. mexicanum* could also occur by the oral route, living *L. vexator* that were potentially infected with *P. mexicanum* sporozoites were force-fed (placed in the rear of the mouth with forceps) to *Sc. undulatus*.

Mosquitoes, collected and maintained as described by Klein et al. (1987) were fed also on *Sc. occidentalis* infected with *P. mexicanum*. Midguts were dissected similarly and examined for oocysts 4–10 days after the initial blood meal.

Blood films of *Sc. undulatus* previously fed on by infected *L. vexator* or force-fed infected sand flies were made at day 0 postexposure (PE) and at 2–4 day intervals 19 days PE. Parasites were counted and parasitemia expressed as the number of parasites per 10,000 red blood cells (RBC). *Plasmodium mexicanum* characteristically occupies all circulating blood cells (Jordan 1970); therefore, the number of infected white blood cells (WBC) per 10,000 red blood cells was also counted. A sufficient number of red blood cells was counted to keep the probable error within 10% according to the method of Gingrich (1932).

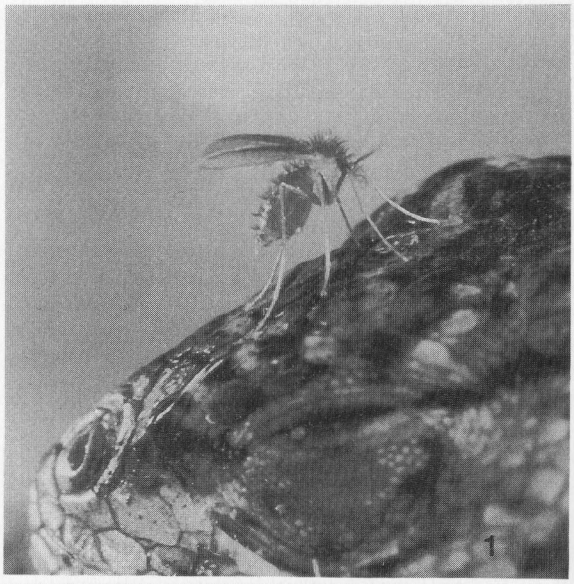
Post-mortem tissue impressions of various organs from lizards infected with *P. mexicanum* were fixed with methanol and stained with Giemsa. In addition, tissues from one lizard (S-51) were fixed in Carnoy's fluid, dehydrated, embedded in paraffin and sectioned at 5–6μm on a rotary microtome. Thin sections were stained with hematoxylin-eosin or Giemsa-colophonium (Bray and Garnham 1962).

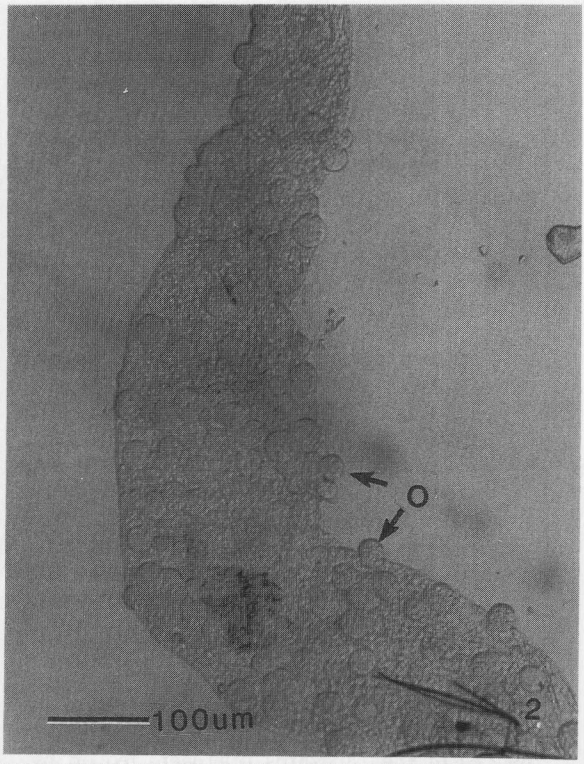
RESULTS

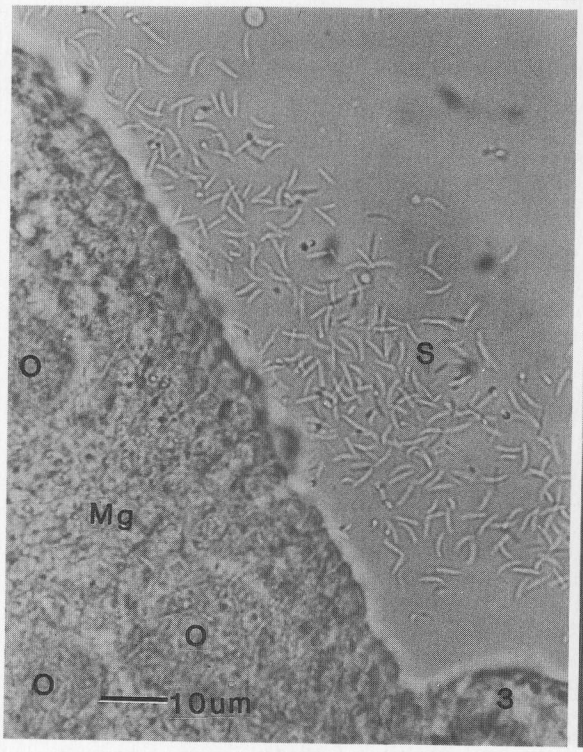
Female *Lutzomyia vexator* readily fed on lizards in the laboratory (Fig. 1). Exflagellation of *P. mexicanum* male gametocytes was observed in blood meals by removing the midgut contents in Ringer's solution within 30 minutes after a female sand fly completed blood feeding. The length of time during which exflagellation occurred was not determined.

The developmental period of *P. mexicanum* in the invertebrate host, *L. vexator*, was relatively short at 27°C and somewhat longer at 24°C. Oocysts were seen first on day 2 PF, often in large numbers (Fig. 2), and developed rapidly at 27°C. Sporoblastoids with budding sporozoites were observed in some oocysts by day 5 PF. Sporozoites were free in the hemocoel by day 6 PF and were present in the salivary glands by day 6.5 PF (Figs. 3, 4). However, when sand flies were maintained at 24°C, sporozoites were not observed in the salivary glands until 8.5–9.0 days PF. All sand flies used in the transmission study were maintained at 27°C and were provided second blood meals on non-infected *Sc. undulatus* subsequent to day 6.5 PF. Oocyst and sporozoite development is reported elsewhere (Klein, et al., in preparation).

Experimental transmission data of *P. mexi-*







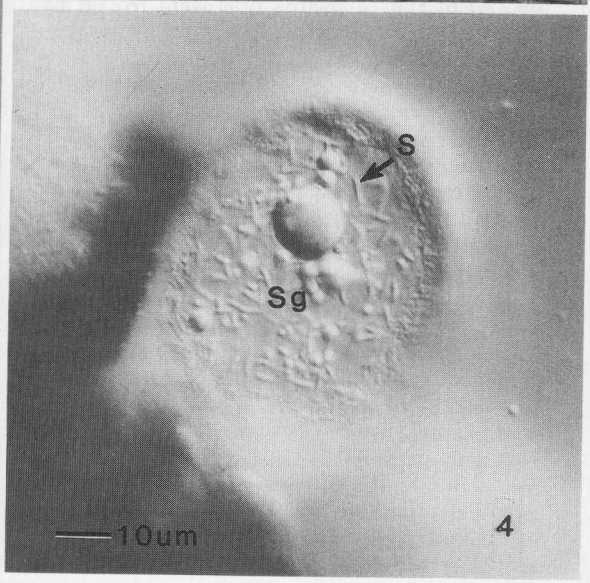


Fig. 1. Female *Lutzomyia vexator* resting on *Sceloporus undulatus* upon which it had previously fed.
Fig. 2. Midgut of *Lutzomyia vexator* with oocysts (0) of *Plasmodium mexicanum*. (Day 5 PF).
Fig. 3. Sporozoites of *Plasmodium mexicanum* (S) from ruptured oocysts (0) on the midgut (Mg) of *Lutzomyia vexator*, day 6 PF on infected *Sceloporus occidentalis*.
Fig. 4. Sporozoites of *Plasmodium mexicanum* (S) in the salivary gland (Sg) of *Lutzomyia vexator* (Nomarski interference contrast).

canum to non-infected *Sc. undulatus* by the bite of infected *L. vexator* female(s) are shown in Table 1. Thirteen *Sc. undulatus* were each fed on by 1–3 *L. vexator* females that had taken bloodmeals from *P. mexicanum* infected *Sc. occidentalis* 7–10 days earlier. All sand flies dis-

Table 1. Laboratory transmission of *Plasmodium mexicanum* to *Sceloporus undulatus* by bites of infected *Lutzomyia vexator* females.

Lizard no.	No. flies fed	Sporozoite rate/(day PF)	Day patent inf.	Day PF lizard died (killed)	Duration patent infection (days)	No. par./ 10,000 RBCs at death	%RBC inf. at death	%WBC inf. at death
S-8	3	+3(7) +3(7) +3(8)	27	52	25	2095	20.3	25.0
S-14[a]	1	+3(9)	26	39	13	930	9.2	10.7
S-15[b]	1	+3(8)	26	47	21	2130	20.2	44.4
S-25[a]	1	+3(9)	33	52	19	2780	25.9	34.3
S-42	1	+3(8)	33	61	28	8270	66.5	55.6
S-43	2	+2(7) +3(10)	26	(66)	(40)	(7122)	(53.0)	(25.0)
S-47	3	+3(8) +3(8) +3(8)	23	(45)	(22)	(3070)	(28.5)	(37.5)
S-50	3	+3(8) +2(9) +3(9)	23	(46)	(23)	(1370)	(12.6)	—
S-51	1	+1(9)	40	96	56	11,960	91.2	24.2
Average[c]			28.6	50.2	27.0	4379	38.9	32.4

[a] Yearling lizard.
[b] Lizard maintained at 27°C.
[c] Lizards which were killed are not included in the average.

sected 0–8 hr after their second blood meal had sporozoites in their salivary glands. Nine (69.2%) of the 13 lizards that were fed on by infected flies showed patent parasitemia with *P. mexicanum*. Two *Sc. undulatus* that were each force-fed more than 5 sand flies which had fed on an infected lizard 10 days earlier did not become infected with *P. mexicanum*.

The F_1 progeny of two species of mosquitoes, *Culex erraticus* (Dyar and Knab) and *Culex territans* (Walker) that were collected in lizard-baited traps, and 107 *Culex apicalis* Adams from a colony at the University of California, Davis also were provided blood meals on *Sc. occidentalis* infected with *P. mexicanum*. None of the *Cx. erraticus* and *Cx. territans* that fed on infected lizards during the same time as the sand flies developed oocysts while all of the *L. vexator* dissected had oocysts (9-54, $\bar{x}$ = 22.1 oocysts). Oocysts were not observed in the *Cx. apicalis* that fed on infected lizards.

Patent *P. mexicanum* infections were observed first in 9 of the experimentally infected *Sc. undulatus* 23–40 days PE ($\bar{x}$ = 28.6 days) (Table 1; Fig. 5). Because lizards were bled approximately every third day, infections may have been patent as early as 2 days prior to positive blood films. The acute infection was allowed to run its course in each of 6 lizards. The remaining 3 lizards were killed after they became anorexic and lethargic; they probably would have survived only a few days longer. The other 6 lizards died of fulminating infections by day 96 PE and became lethargic and anorexic several days prior to death. Force-feeding 2 lizards in an effort to keep them alive was not successful. The survival period varied from 13 to 56 ($\bar{x}$ = 27.0) days following the detection of parasites in the blood film and 39–96 ($\bar{x}$ = 57.8) days PE. Two of the longest surviving lizards, S-42 and S-51, were adult females. Both lizards deposited abnormal and infertile eggs during the course of the infection.

The number of parasites per 10,000 RBC, percent of infected RBC, and percent of infected WBC (per 10,000 RBC) on the day prior to death are shown in Table 1. It was difficult to prepare blood films during the later course of the infections due to anemia. Excluding lizards S-42 and S-51, which had approximately 4× and 6× the number of parasites, respectively, as the other 4 lizards which died, the mean parasite count at death was 1,983 (19.8%). The proportion of infected RBC approximated the percent parasitemia at levels below 25%. However, as parasitemia increased, the number of multiply infected RBC also increased, as shown by S-42 which had 82.7% parasitemia, but only 66.5% of the RBC infected (Table 1; Figs. 6, 7). Although the parasitemia of S-51, as expressed in numbers of parasites per 10,000 RBC did not increase significantly during the latter part of the infection (11,420, day 89 PE to 11,960, day 95 PE), the proportion of infected RBC continued to increase rapidly until nearly every RBC was parasitized (72.4–91.2%) (Figs. 6, 7).

The transformed (Y = Log number of parasites per 10,000 RBC) course of infection and

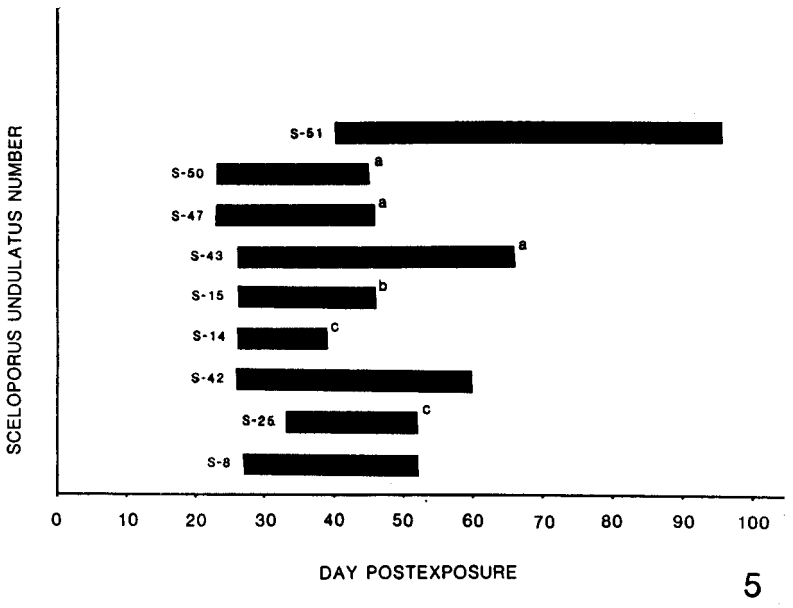


Fig. 5. Prepatent and patent periods of *Plasmodium mexicanum* infection and survival time of individual *Sceloporus undulatus* infected by bites of *Lutzomyia vexator*. [a]Killed during the course of the infection. [b]Maintained at 27°C. [c]Yearling lizards.

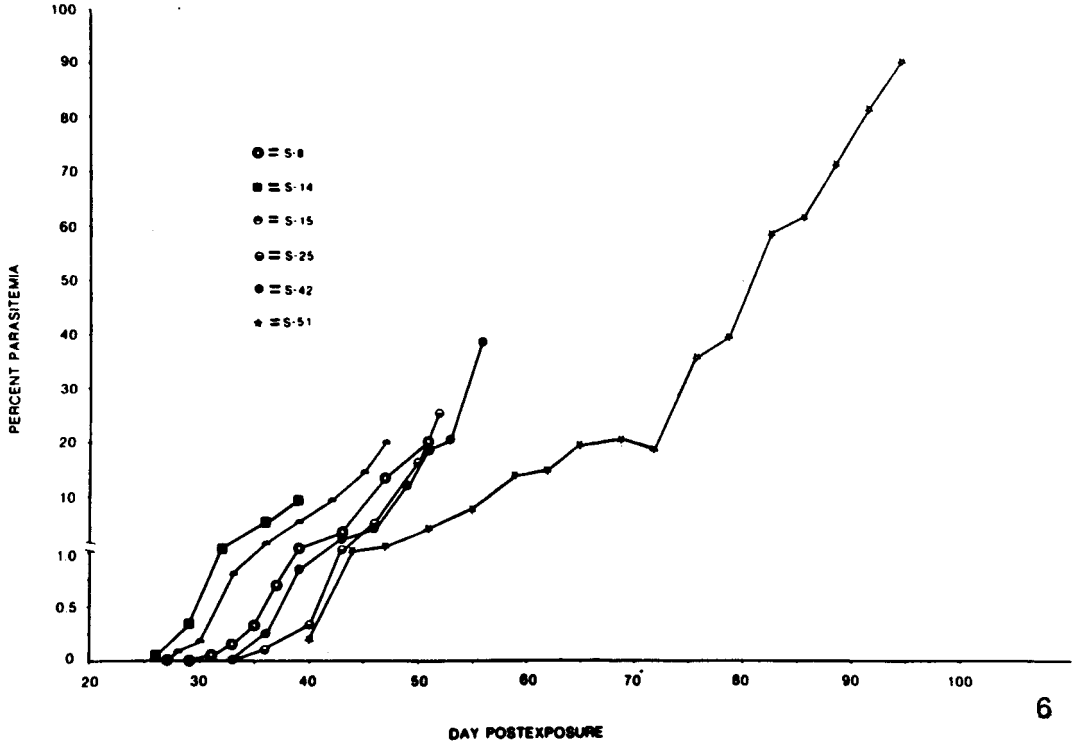


Fig. 6. Percent of infected RBCs during the course of *Plasmodium mexicanum* infection for 6 *Sceloporus undulatus* infected by bite of *Lutzomyia vexator*.

linear regression analysis is shown in Fig. 7. The progression of the acute infection can be explained as an exponential linear relationship for parasitemia levels of fewer than 500 parasites/10,000 RBC (5% parasitemia). In general, the slopes of the acute infections were similar in all lizards with less than 5% parasitemia. Except for lizards S-47, S-51 and S-43, the slopes of

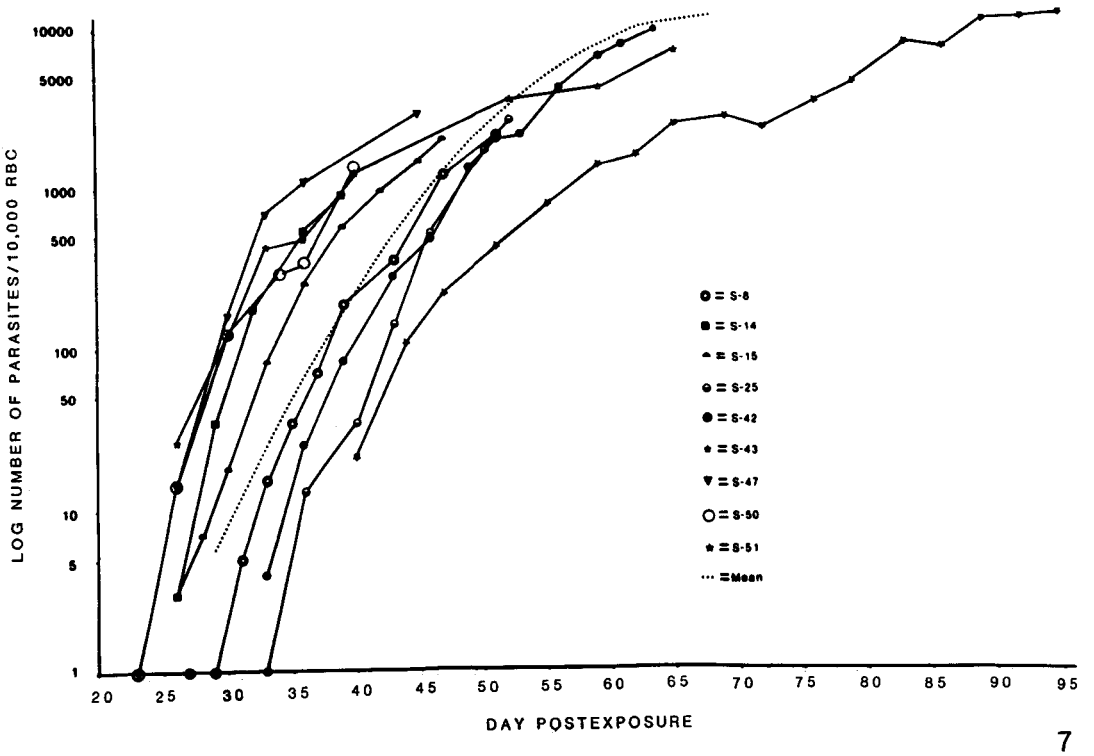
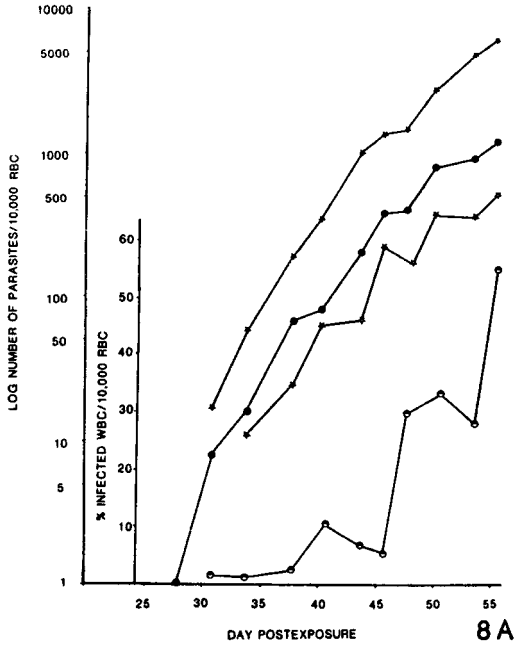
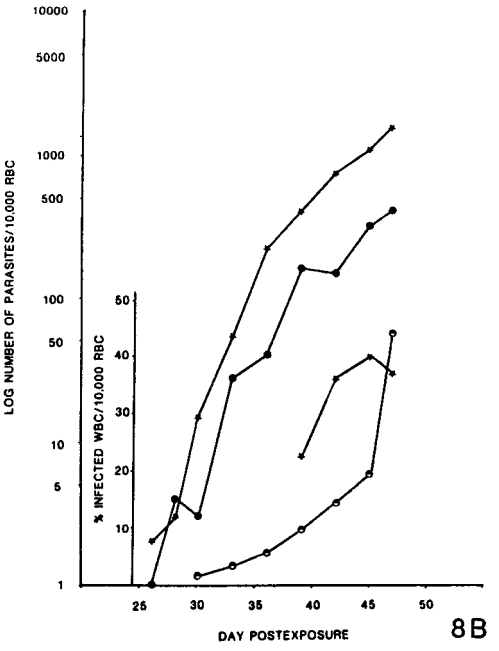


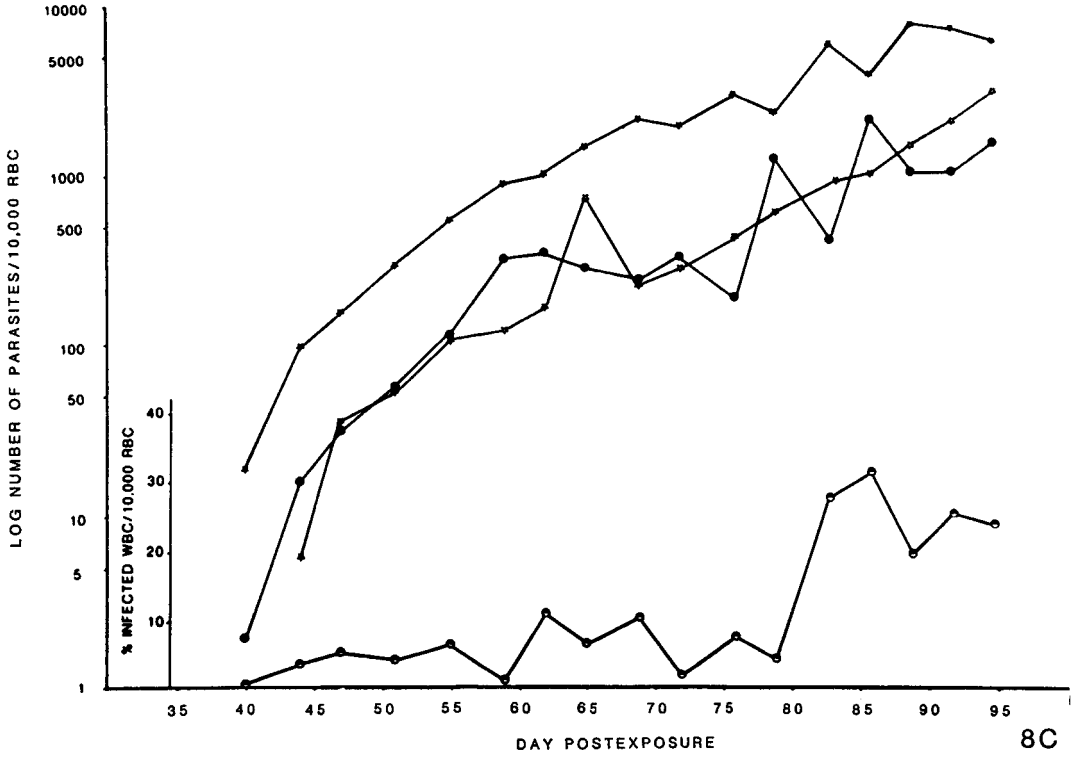
Fig. 7. Course of acute infection of *Plasmodium mexicanum* in 9 *Sceloporus undulatus* infected by bite of *Lutzomyia vexator*. The predicted parasitemia ($R^2 = 0.88$) during the course of an "average" infection is shown by the dotted line.

parasitemia increase were not significantly different ($P = 0.01$). Lizards S-42, S-43 and S-51 had the lowest rate of increase in parasitemia, survived the longest, and had terminal parasitemias of >70% (71.2, 87.7 and 119.6%, respectively). After the parasitemia reached >500 parasites per 10,000 RBC, the rate of increase was reduced and followed a quadratic relationship. When considering all lizards that died or were killed (and probably would have died within a few days), there were significant differences ($P = 0.01$) in the curves of the quadratic equation. However, the curves were similar enough to average for all lizards (Fig. 7). A regression of parasitemia over the course of the infection for all lizards was performed ($R^2 = 0.88$). The initial positive blood film (patent infection) was adjusted to begin on the mean day of patent infection (28.6) since we were interested in the average course of infection. The number of trophozoites, schizonts, and gametocytes in RBC and percent of WBC infected with various stages of *P. mexicanum* during the course of infection in lizards that died of fulminating infections is shown in figures 8A–8C. Both immature (single nucleated parasites larger than but not displacing the host cell nucleus) and "mature" gametocytes (as described by Garnham 1966) are included together. The number of trophozoites increased logarithmically during the course of the infection. In general, the numbers of schizonts and gametocytes also increased logarithmically, but appeared to show more variation, partially due to sampling error, because fewer numbers of schizonts and gametocytes were observed per 10,000 RBCs. From the limited numbers of blood films, it appears that schizogony occurred at roughly 3–4 day intervals. The percentage of infected WBC and thrombocytes generally increased as the number of infected RBC increased (Figs. 8A–8C). However, the relatively low and variable number, coupled with the possibility that white blood cells may have ruptured during the preparation of a blood film increased the potential for error in the estimation of infected WBCs.

Numerous parasites were observed in lymphocytes of spleen tissue impressions prepared less than 2 hr after death of lizards. Immature erythrocytes and lymphocytes from smears of bone marrow extracts were also infected with *P. mexicanum* parasites. However, the number of parasites observed in the spleen tissue impressions was much greater and this organ appears to be the primary site of attack. Schizogony was also observed in the endothelial cells of the brain capillaries in some lizards. Parasites were not found in the lung, liver, kidney, intestine, pan-







Figs. 8A–8C. Number of *Plasmodium mexicanum* trophozoites (★), schizonts (●), and gametocytes (☆) per 10,000 RBC, and % of infected WBC (◓) during the course of infection for 3 *Sceloporus undulatus* (S-42, A; S-15, B; and S-51, C) infected by bite of *Lutzomyia vexator*.

creas, heart or uterus tissues of 5 of the lizards. Occasionally, cells of the above tissues appeared to be infected, but it could not be determined if the parasites were external, possibly from ruptured WBC or intracellular, since fixed or circulating lymphocytes within these tissues often had numerous parasites. Thin sections of these tissues from lizard S-51, also failed to reveal intracellular parasites.

DISCUSSION

Our data demonstrate conclusively that *P. mexicanum* can be transmitted from *Sc. occidentalis* to *Sc. undulatus* by bite of the sand fly *Lutzomyia vexator*, a species that coexists with the parasite in California. Progeny of two species of mosquitoes, *Culex erraticus* and *Cx. territans*, which were collected in lizard-baited traps, failed to transmit this parasite, while as many as 50 oocysts developed on the midgut of *L. vexator* feeding on the same lizard. *Culex apicalis*, which feeds on reptilian blood, similarly did not develop oocysts. This provides further evidence that sand flies are the natural vectors of *P. mexicanum*, especially since sporogony and transmission of *P. floridense* has been demonstrated for *Cx. erraticus* (Klein et al. 1987).

The transmission rate of *P. mexicanum* by bite of *L. vexator* is relatively high and compares well with that of some other malarias (Coatney et al. 1945, Russell and Mohan 1942). Transmission occurred in 62.5% (5/8) of the *Sc. undulatus* which were fed on by one sand fly and 80% (4/5) of the *Sc. undulatus* which were bitten by 2 or more sand flies. After a bloodmeal, residual sporozoites were occasionally observed in the mouthparts of dissected sand flies, but only in small numbers (<5).

The development of *P. mexicanum* in laboratory-reared *L. vexator* maintained at 27°C is rapid, with sporozoites observed in the salivary glands by day 6.5 after feeding. Sporozoites were not observed in the salivary glands until day 8.5–9.0 PF for sand flies maintained at 24°C. Ayala and Lee (1970) indicated that sporozoites from laboratory-infected, wild-caught sand flies were not observed in the hemocoel until days 11–14 PF when maintained at room temperature (24–26°C). Differences observed in the development period of *P. mexicanum* at similar temperatures in Ayala and Lee's (1970) and our studies are not understood, but may be related to the different origin of either sand fly or parasite strains. Maximum and minimum temperatures when sporozoite development ceases were not determined.

Vanderberg (1975) showed that sporozoites require a period of maturation after their release from the oocyst and that *P. berghei* sporozoites in the salivary glands of a mosquito are 10,000 times more infective than sporozoites from the oocyst of the same mosquito. However, once released from the oocyst, the development of infectivity appears to be time-dependent rather than site-dependent, (i.e., in some cases, hemocoel sporozoites and salivary gland sporozoites are equally infective). Only one lizard was fed on by an infected sand fly on day 7.0–7.5 after its initial bloodmeal. It did not become infected. The sand fly was dissected within 8 hr following the second blood meal, and had more than 100 sporozoites in the salivary glands. Nine of the 12 (75%) remaining lizards, which were each fed on by 1–3 infected sand flies 8–10 days after their initial blood meal on infected *Sc. occidentalis* became infected, indicating that sporozoites of sand flies maintained at 32°C are infective within 8 days of feeding (Table 1).

Both attempts to transmit *P. mexicanum* by ingestion of whole infected sand flies were unsuccessful. Transmission by oral ingestion of sporozoites has been reported to be occasionally successful under certain laboratory conditions for malarias transmitted by mosquitoes (Shortt and Menon 1940, Young 1941, Porter et al. 1952, Yoeli and Most 1971). The oral route may be the mode of transmission for *P. agamae* (Petit et al. 1983). But because sporozoites are quickly killed in acid concentrations similar to that found in the gut, it is believed that oral transmission will only occur if the sporozoites penetrate the tissues of the mouth and throat. Although hatchling lizards may eat sand flies, unrestrained yearlings were rarely observed feeding on sand flies. Mature *Sc. occidentalis* and *Sc. undulatus* were never observed feeding on *L. vexator* in the laboratory. Since lizards do not normally masticate ingested flies and both attempts to orally transmit *P. mexicanum* failed, transmission by the oral route, if it occurs, probably has little epidemiological significance.

Prepatent periods in experimentally transmitted *P. mexicanum* by bite of infected *L. vexator* ranged from 23 to 40 ($\bar{x}$ = 28.6) days. In examining natural infections of another lizard malaria, *P. floridense*, Goodwin (1951) showed that parasites were not observed in blood films until approximately 2 weeks after the wild-caught lizards were collected. In another study, parasites were not observed in the blood film of one lizard until 27 days after capture (Goodwin and Stapleton 1952). Present studies on the transmission of *P. floridense* indicate that the prepatent period is affected by temperature and is more than 20 days at 18–24°C for bite induced and IP induced infections. However, when lizards were maintained at 32°C, the prepatent

period was reduced by as much as 7 days (Klein et al., in preparation).

Recent studies on other hemosporozoans, *Schellackia golvani* and *Schellackia occidentalis*, also indicate that temperature affects the length of the prepatent period (Klein et al., in preparation). For lizards maintained at room temperature (18–24°C), *Schellackia* sporozoites were not observed in blood films until day 21 and 37 postingestion, respectively. However, when lizards were maintained at 32°C (90°F), sporozoites were seen in the blood films as early as day 10 and 7 postingestion, respectively. These studies and those by Goodwin and Stapleton (1952) (assuming that the naturally acquired infections were not relapses), and other studies on *P. mexicanum* laboratory transmission, support the hypothesis of a lengthy prepatent period for at least two of the saurian malarias and other hemosporozoa of lizards. In addition, Thompson and Winder (1947) observed that parasitemias increased at a faster rate in blood-inoculated lizards that were maintained at higher temperatures. The effects of temperature and the normal range of host temperature in relation to malaria parasites still require investigation.

In general, lizards that were fed on by more than one infected sand fly developed earlier patent *P. mexicanum* infections. One lizard (S-51), fed on by only one sand fly, in which fewer than 5 sporozoites were observed in the salivary glands and around the head within 8 hr after the second blood meal did not develop a patent infection until 40 days after feeding. Ayala (1971) showed that *Sc. occidentalis* inoculated with sporozoites from 5 sand flies (some having more than 100 oocysts on the midgut) had a prepatent period of 21 days. Observations on human, rodent and avian malarias show that, within limits, the higher the inoculum of sporozoites, the shorter the prepatent period (Boyd 1940, Greenberg et al. 1950). Based on the criterion used in the present studies, i.e., the number of sporozoites remaining in the salivary glands and head region following feeding, the length of the prepatent period appears to be in part dependent on the number of sporozoites. However, host temperature maintenance in relation to parasite development may also play an important role in the early course of the infection.

Natural infections of *P. mexicanum* occur in both *Sc. occidentalis* (California) and *Sc. undulatus* (Wyoming) (Ayala 1971, Greiner and Daggett 1973). Separate studies indicate that both species are highly susceptible to *P. mexicanum* and often die of fulminating infections during the acute phase (Ayala 1971, Jordan 1970, Thompson and Huff 1944, Thompson 1944). The age of the lizard (*Sc. occidentalis*) also appears to have a significant effect on the course of the infection. When hatchling lizards (3–5 months old) were blood-inoculated with *P. mexicanum*, all lizards died of fulminating infections, but only 3/10 wild caught and naturally infected yearling *Sc. occidentalis* died (Ayala 1971). However, the course of the infection for blood-inoculation of some malarias is often more severe than sporozoite inoculation. In the present studies, 2 yearling and 7 mature *Sc. undulatus* collected in Florida were experimentally infected by bite of *L. vexator*. Six of the lizards (including the 2 yearling lizards) died of fulminating infections within 96 days after exposure. The other 3 lizards were killed when it became evident they were about to die, at parasitemias ranging from 20 to 75%.

Parasitemias of *P. mexicanum* ranged from 930 (yearling) to 11,960 (mature female)/10,000 RBC at the time of death (Table 1, Fig. 7). Maximum parasitemias attained by *P. mexicanum* from previous studies ranged from 4,100 to 8,100 for *Sceloporus olivaceus* Smith, 2,812 for *Sc. undulatus undulatus*, and 2,750 for *Sc. undulatus consobrinus* (Thompson and Huff 1944, Thompson 1944). Maximum parasitemias for unnatural hosts, *Phrynosoma cornutum* (Harlan) and *Crotaphytus collaris* Say only reached 392 and 238, respectively (Thompson and Huff 1944). Results of the present studies are similar to those of Thompson and Huff (1944) and Thompson (1944), except that several of the *Sc. undulatus* which were blood-inoculated in the previous studies did not develop fulminating infections. The higher parasitemia in some lizards in the present study may be a result of a larger sample size of lizards.

As indicated by Fig. 7, it appears that lizards which survive for a longer period of time (>25 days) subsequent to patent infection, develop parasitemias at a slower rate (slope <0.40). However, these infections were also observed over a longer period of time, with the result that higher parasitemias might therefore have developed (>70%). Although the curves of the transformed course of infection appear to be similar, there are significant differences between some of the curves. These differences may be attributed to age and sex of the lizards (adult females surviving the longest), host immune response to the parasite, adaptation to a laboratory environment, temperature (behavioral orientation to the light source), length of time surviving patent infection, number of sporozoites inoculated during feeding, and other possible factors.

Thompson and Huff (1944), suggest that variations in the course of infection, gametocyte production, and cellular distribution of *P. mex-*

icanum parasites are due to host differences rather than alteration of parasites. They found that *P. mexicanum*, a natural parasite of certain *Sceloporus* species, lost its gametocytes when transferred by blood-inoculation to another lizard, *Crotaphytus collaris*. However, gametocyte production resumed upon experimental passage of the parasite to a third host species, *Sc. olivaceous*. In the present study, "mature" gametocytes were observed in all lizards except S-14 and S-15. *Lutzomyia vexator* that fed on S-51 and S-42 on days 11 and 21, respectively, following the detection of parasites, and when mature gametocytes were present in the blood, developed low numbers of oocysts. Sporozoites developed normally and appeared viable, but were not injected into another lizard to determine infectivity. It was not determined if gametocyte production would be increased during the chronic phase of the infection since all lizards died (or were killed) during the acute phase.

As in studies by Thompson and Huff (1944) and Jordan (1970), it was observed that *P. mexicanum* parasites primarily invade erythrocytes and lymphocytes of *Sc. undulatus*. Thrombocytes were less frequently invaded. No attempt was made to distinguish between granulocytes and macrophages. Determining the percentage of lymphocytes infected was often difficult since occasionally many of the cells ruptured in blood film preparation, especially during the latter part of the acute phase. These results agree with those of Thompson and Huff (1944) who observed that 93% of the circulating cells infected with *P. mexicanum* were in erythrocytes, with a small percentage in lymphocytes and thrombocytes.

Tissue impressions of the spleen and bone marrow revealed numerous asexual forms of *P. mexicanum* in lymphocytes. Although exoerythrocytic (EE) forms were often seen in lymphocytes (Jordan 1970, Thompson and Huff 1944), they were rarely observed in other tissues in *Sc. undulatus*. However, in unnatural hosts such as *Phrynosoma cornutum* and *C. collaris*, *Plasmodium mexicanum* EE forms were frequently observed in fixed connective tissue while occurring less frequently in the circulating cells (Thompson and Huff 1944). When studying fixed tissues, Jordan (1970) recovered schizonts and segmenters from impressions of internal organs, especially endothelial cells of the brain capillaries. In our study, impressions of the brain of some lizards also demonstrated schizogony in the endothelial cells. Endothelial cells were heavily infected in some lizards. However, attempts to identify parasites in liver, lung, intestine, pancreas, heart and uterus from tissue impressions of 4 *Sc. undulatus* were unsuccessful.

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