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## EXPERIMENTAL INOCULATION OF PANAMANIAN MAMMALS WITH *LEISHMANIA BRAZILIENSIS*\*

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**ABSTRACT:** Attempts to infect native Panamanian mammals with culture forms of local human strains of *Leishmania braziliensis* produced cutaneous infections for the first time in the spiny rat (*Proechimys semispinosus*), white-tailed tree rat (*Tylomys panamensis*), kinkajou (*Potos flavus*), and olingo (*Bassaricyon gabbii*). While infections of hamsters and cotton rats have usually lasted throughout the life of the animals, the parasites disappeared from the spiny rat and tree rat lesions within a month, but persisted in the kinkajous for at least 2 and 3 months.

For a number of years we have attempted, at this laboratory, to infect native mammals with human strains of *Leishmania braziliensis*, *sensu lato*, in order to discover potential reservoir hosts of leishmaniasis. Early attempts here to infect wild mammals met with failure, but the routine inoculation of golden hamsters and occasional trials with cotton rats from our breeding colonies have shown that these rodents can be readily infected. Although cotton rats of the same species, *Sigmodon hispidus*, occur in Panama they are not primarily forest animals and so their susceptibility can have only limited significance in the epidemiology of this forest disease. The present paper reports the results of inoculation experiments conducted between June 1963 and September 1964.

Lainson and Strangways-Dixon (1964) carried out inoculations of wild animals with *Leishmania mexicana* in British Honduras. They obtained infections in cotton rats, but of 41 specimens of other wild mammals, representing 11 species, only a single common opossum (*Didelphis marsupialis*) gave positive results. No lesion was produced at the site of inoculation and the infection was demonstrated only by culture from the liver.

### MATERIALS AND METHODS

There is always the possibility that wild-caught mammals are already immune as a result of natural exposure in the forest. To avoid this difficulty, animals born or reared in the laboratory were used whenever available. In 1963 the breeding of the

arboreal white-tailed rat (*Tylomys panamensis*) was initiated at this laboratory. Although the average litter size was found to be only two, and the gestation period about 35 days, it has now been possible to build up the colony to about 130 individuals. Most of the *Tylomys* used in this study were laboratory-bred from wild-caught parents. The spiny rats (*Proechimys semispinosus*) were born in the laboratory from wild-caught gravid females. The woolly opossums (*Caluromys derbianus*) were laboratory-reared, but were brought into the animal room in the marsupial of wild-caught females. The cotton rats (*Sigmodon hispidus*) were from our colony established several years ago with stock from commercial sources in the U. S. The other animal species were wild-caught. Most of the live-trapped mammals were obtained near the town of Achioté, Province of Colon, Panama, where human cutaneous leishmaniasis has been known to be endemic for many years.

The two strains of *Leishmania* used in the present study were isolated in Senekjé's modification of NNN culture medium from the cutaneous lesions of patients. The first of these isolates (M-strain) was obtained in December 1960 from a patient having multiple lesions. The patient was a 48-year-old male who came to the laboratory for treatment. He had spent most of his life near Concepción, in Chiriquí Province, but had developed the lesions while on the Changuinola River in Bocas del Toro Province, a known endemic area. The patient reported that the first lesion had started on the back of his hand in October 1960, and that about 3 weeks later others began to appear. When seen at the laboratory, the patient had an ulcer of from 1 to 3 cm in diameter in each of the following locations: hand, arm, tip of nose, thigh, neck, and left ear. This strain has been maintained in culture and in hamsters.

The other strain used in this study (VH-strain) is of more recent origin. In April 1964 one of our fieldmen developed a large lesion on his upper left arm while engaged in trapping mammals in the Achioté area. This man has worked in the field for the Gorgas Memorial Laboratory for 28 years, and had been exposed in endemic areas many times without previously contracting leishmaniasis. Treatment was initiated as soon as the condition

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TABLE I. Inoculations of Panamanian mammals with two human strains of *L. braziliensis*.

	M-strain				VH-strain			
	No. inoculated	No. with L-D bodies	Duration of L-D bodies in days	No. showing swelling	No. inoculated	No. with L-D bodies	Duration of L-D bodies in days	Duration swelling in days
<i>Caluromys derbianus</i> Waterhouse (woolly opossum)	4	0	—	0	3	0	—	0
<i>Didelphis marsupialis</i> L. (common opossum)	0	—	—	—	2	0	—	0
<i>Sigmodon hispidus</i> Say and Ord <sup>1</sup> (cotton rat)	2	2	167+	2	2	2	73+	73+
<i>Tylomys panamensis</i> Gray (white-tailed rat)	12	0	—	3	16	4	13-24	50
<i>Proechimys semispinosus</i> (Tames) (spiny rat)	1	0	—	0	2	1	20	47
<i>Dasyprocta punctata</i> Gray (agouti)	0	—	—	—	2	0	—	0
<i>Potos flavus</i> (Schreber) (kinkajou)	5	1	90	3	2	1	54	54
<i>Bassaricyon gabbii</i> Allen (olingo)	0	—	—	—	1	1	28	46
Totals	24	3		8	30	9		

<sup>1</sup> These animals were from our breeding colony, started with stock from the U. S., but the same species is native to Panama.

was diagnosed, and no additional lesions developed. The single ulcer proved to be unusually resistant to drug therapy, however. The isolate used in the present study was obtained immediately before treatment was started.

The experimental animals were used in groups of from one to four, and each group was injected intradermally with leptomonads from cultures of the same age. Two hamsters were used as controls for each group and were similarly inoculated. All animals were injected in the side of the foot and on the tail, and some were also inoculated on the tip of the nose. The animals were examined after 2 or 3 weeks, and when a swelling was observed, smears were taken. Similar subsequent examinations were made monthly thereafter. Smears were fixed in Carnoy's solution and Giemsa stained. Impression smears and cultures were made from the heart, liver, spleen, lungs, and kidneys of animals that died during the experiment.

## RESULTS

The hamster controls all became positive with both the M- and VH-strains. L-D bodies could be found near the sites of inoculation after about 3 weeks, and these animals remained positive throughout the experiment. The lesions were usually marked by prominent swellings.

The results of the inoculations with the two strains are shown in Table I. With the M-strain it can be seen that of the 24 animals, representing five genera, the only one, other than the cotton rats, from which L-D bodies were

recovered was an adult kinkajou (*Potos flavus*). This animal was inoculated on 24 October 1963, and on 21 January 1964 (90 days later) a slight swelling containing L-D bodies was found on the tail at the site of the injection. Smears from the inoculated foot were negative, however. Unfortunately, this kinkajou died of undetermined causes 3 weeks later. At autopsy the tail appeared normal and no parasites could be demonstrated in smears or cultures.

The VH-strain proved infective for a greater variety of animals. Out of 30 native mammals, representing eight genera, lesions with L-D bodies were produced in nine animals of five genera. It was not possible to demonstrate L-D bodies in any of the five marsupials nor in the two agoutis. Several of the other rodents became positive, but only the cotton rats had long-lasting infections. One of the two inoculated kinkajous developed an infection, but unfortunately this was not apparent until the animal died 54 days after inoculation and L-D bodies were found in the tail at autopsy. Hematoxylin-eosin-stained sections revealed moderate numbers of L-D bodies in the outer epithelial layers of the tail but none in the internal organs.

At the time of these experiments, only a single olingo (*Bassaricyon gabbii*) was available. Since the olingo is a close relative of the kinkajou, it seemed of some significance

TABLE II. Inoculations of the white-tailed tree rat, *Tylomys panamensis*, with VH-strain of *L. braziliensis*.

<i>Tylomys</i>	Sex	Age	Date of inoculation 1964	Date L-D bodies recovered	Date last visible swelling
No. 516	♂	3 mos	4 June	23 June	15 Sept.
517	♂	2½ mos	4 June	25 June	23 July
521	♀	11 wks	11 June	—	—
522	♂	11 wks	11 June	23 June	15 Sept.
524	♀	3 mos	14 July	—	23 July
526	♀	3 mos	14 July	—	23 July
23	—	—	28 July	—	—
29	—	—	28 July	—	—
14	—	—	28 July	—	—
19	—	—	28 July	—	—
541	—	2 wks	28 July	—	—
542	—	2 wks	28 July	20 Aug.	15 Sept.
531	—	4 wks	28 July	—	—
532	—	4 wks	28 July	—	—
547	—	7 wks	17 Sept.	—	—
548	—	7 wks	17 Sept.	—	—

that L-D bodies were found in smears from the tail of this animal 28 days after injection. It was not possible, however, to find L-D bodies subsequently even though a slight swelling on the tail persisted for at least 46 days.

Since white-tailed tree rats of known ages were available in our breeding colony, an attempt was made to determine whether age or sex might influence susceptibility to *Leishmania* infection. The 16 *Tylomys* inoculations with the VH-strain summarized in Table I are shown in detail in Table II. The 12 animals numbered in the 500-series were all laboratory-born while the other four were wild-caught. From this table it would appear that age is probably not an important factor. Although one suckling rat 2 weeks old developed an infection, several other young animals did not. Some young adults 2½ to 3 months old developed infections, but others did not. It may be of significance, however, that within the laboratory-born group of six males and six females, no females developed infections while four of the six males did. Four wild-caught males of unknown ages failed to show infections, but these animals had been trapped in an endemic area and therefore could have been naturally exposed to the parasite. Experiments to explore further the possibility of sex-resistance in *Tylomys* are presently under way.

In each of the four *Tylomys* that developed infections, L-D bodies were recovered only once. They were found in smears made from the tail between 13 and 24 days after inoculation, but swellings remained visible on the tails

of these animals for as long as 3 months. No parasites were found in smears from the inoculated feet of the same animals.

#### DISCUSSION

In comparing the M-strain and VH-strain inoculations, it is apparent that the latter gave a higher percentage of positives among native Panamanian mammals. This variation may have been caused by a primary physiological difference between the two strains, but more probably it reflects a gradual loss of virulence by the M-strain as a result of passage in hamsters and cultures during several years. No differences in virulence between the two strains were apparent, however, with respect to inoculations of golden hamsters and cotton rats.

In hamsters and cotton rats the *Leishmania* infections usually last until the death of the animal. In contrast, the experimental infections in native rodents were rather transitory and L-D bodies were not recovered more than 1 month after inoculation. Two positive kinkajous gave longer-lasting infections of approximately 2 and 3 months, respectively.

It is believed that this is the first report of experimental cutaneous *Leishmania* infections in the spiny rat, white-tailed tree rat, kinkajou, and olingo.

#### LITERATURE CITED

- LAINSON, R., AND J. STRANGWAYS-DIXON. 1964. The epidemiology of dermal leishmaniasis in British Honduras. Tr. Roy. Soc. Trop. Med. Hyg. 58: 136-153.