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THE BEHAVIOUR OF A LIZARD LEISHMANIA IN HAMSTERS AND BABY MICE

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SUMMARY

Leishmania adleri Heisch, a lizard Leishmania from Kenya parasitic in *Latastia longicorda revoili*, was found to produce transient cryptic infections in 6 out of 24 adult and 6 out of 11 baby hamsters inoculated intrasplenically or intraperitoneally. The infection lasted up to 5 weeks and could be detected only by culturing material from the spleen.

Forty four out of 71 baby mice inoculated intradermally became infected. In 19 the infection was patent in the subcutaneous connective tissue. No patent infections were found later than 10 days after inoculation. After this period the infection was cryptic and could be demonstrated by culture of material from subcutaneous tissue.

Leishmania adleri has antigens in common with all the human Leishmanias. The relationship between mammalian and lizard Leishmanias is discussed.

INTRODUCTION

Leishmania sp. have been recorded from the following genera of lizards in the Old World: *Agama, Tarentola, Latastia, Hemidactylus, Gymnodactylus* and *Chameleo*. In the first five of the above genera Leishmaniasis is a visceral infection; the parasites which can apparently persist only in suitable sterile media, can be isolated by culture of heart's blood. *Leishmania chameleonis* on the other hand is a parasite of the cloaca where it lives as a flagellate together with a rich flora. FRAENKEL [4] observed one case of invasion of the epithelium lining the cloaca.

Up to the present one species of *Leishmania* has been recorded from the New World viz. *Leishmania henrici* (LEGER, 1918) from a lizard of the genus *Anolis* in Martinique. *L. henrici* occurs in the rectum in the form of a leptomonad and occasionally invades the blood stream where both flagellates and L.D. bodies have been recorded.

L. chameleonis and *L. henrici* are of theoretical interest in so far as they represent a primitive stage in the evolution of the *Leishmanias*. The transmission of these two species has not been studied but since they both multiply in the posterior part of the alimentary tract it is reasonable to assume that they are transmitted by coprophagous insects which are eventually ingested by the vertebrate host.

Adaptation to internal organs and blood stream of lizards was a later stage in the evolution of the Leishmanias. This adaptation permitted both transmission by bite of insect vectors as well as their ingestion by insectivorous reptiles. At this stage of their evolution the *Leishmanias* became a-flagellar intracellular parasites in their vertebrate hosts, but reverted to the original active flagellar forms outside their vertebrate hosts, i.e., in cultures and in their insect vectors (*Phlebotomus minutus* in the case of *L. tarentolae* and probably *P. clydei* in the case of *L. adleri*). Finally, a lizard *Leishmania* became adapted to a mammalian host via a

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Phlebotomus sp. which fed both on reptiles and mammals. The above scheme of the evolution of the Leishmanias first proposed in outline by LEGER for trypanosomes and discussed by ADLER [1] and HOARE [6] implies a phylogenetic relationship between the Leishmanias of mammals and those of lizards. This supposition is justified not only by similarities in morphology and life histories (as far as they are known) between the visceral Leishmanias of lizards and the Leishmanias of mammals, but also by the presence of antigens common to both groups. Thus *L. adleri* has antigens in common with *L. infantum, L. brasiliensis* and *L. tropica.* It is interesting to note that there is more cross-agglutination between *L. adleri* and *L. infantum* than between *L. infantum* and *L. tropica* and sera prepared against *L. adleri* gave a higher titre against *L. brasiliensis* than against *L. tropica* (1 in 800 as against 1:50). The sera were tested by their effect on the growth of homologous and heterologous species on media in which the immune sera were incorporated.

The discovery of *L. tarentolae*, Wenyon, 1921, by SERGENT *et al.* (1914) raised a suspicion that the gecko *Tarentola mauretanica* may act as a reservoir of *Leishmania tropica.* We were impressed by the presence of antigens common to *L. adleri* and the human Leishmanias, particularly the visceralising species, and thought it interesting to test the behaviour of the former in hamsters and baby mice.

MATERIAL AND METHODS

Two strains of *L. adleri* kindly provided by Dr. R. B. Heisch of the Medical Research Laboratory, Nairobi, were used in these experiments. In addition, strains isolated from hamsters and mice inoculated during the course of this work were also used. There were no significant differences in the results obtained with the different strains. Experiments on hamsters were carried out between 1/7/54 and 28/11/58. Twenty four adult hamsters were inoculated, 20 intrasplenically and 4 intraperitoneally, and in addition 11 baby hamsters (7 days old) were inoculated intraperitoneally. The adults were laparatomized at various intervals and fragments of the spleen were removed for culture and for microscopic examination of Giemsa stained material. In no single instance were parasites seen in stained material but 6 out of the 24 adults were found positive by cultures from spleens on semi-solid Locke-blood-agar; of these one had been inoculated intraperitoneally and 5 intrasplenically. The positive animals were examined from 6 to 37 days after inoculation. No cultures were obtained from animals examined more than 37 days after inoculation. Of the 11 baby hamsters inoculated intraperitoneally 6 were found positive by cultures made from the spleen; in no case were parasites found by direct microscopic examination. These 6 positive animals were examined 14 to 28 days after intraperitoneal inoculation.

A total of 71 baby mice from 12 litters were inoculated intradermally with rich cultures together with suspensions of Indian ink by the method described by ADLER & HALFF [2]. The litters were from 1 to 8 days old at the time of inoculation. We observed no significant differences in the final result which could be attributed to age differences in the baby-mice at the time of inoculation. The animals were sacrified at various intervals, and preparations were made from the connective tissue of the dermis, and stained with Giemsa. In addition cultures were made from the subcutaneous tissue and from the spleen.

Out of the total of 71 animals 19 were found positive by direct microscopic examination. The parasites were found in connective tissue cells, to a lesser extent in large mononuclears. In no case were animals found positive by direct microscopic examination later than 10 days after inoculation. Granules of Indian ink were also found in connective tissue cells and large mononuclears. It is interesting to note that granules of Indian ink are first uniformly distributed in connective tissue cells but later form conglomerations in the protoplasm. Cultures made from the subcutaneous tissue were positive in 44 cases, i.e., more than half of the positive cases could not be detected by microscopic examination. A culture from subcutaneous tissue was obtained in one case 36 days after inoculation and all cultures made after this period were negative. Cul-

tures from the spleen were obtained, in one case only, 7 days after inoculation.

Ten of the baby mice had been inoculated with cultures grown on Locke-Serum-agar containing 1:5 specific immune serums. In media into which immune serum is incorporated the type of growth of *L. adleri* depends on the concentration of immune serum. In the higher concentrations discrete colonies of a-flagellar forms together with forms showing incompletely developed flagella are produced. As the concentration of immune serum diminishes clumps of active flagellates appear. As with other Leishmanias the highest concentrations of high titre sera do not inhibit growth of the organisms in vitro — they merely change the type of growth. The results in baby mice inoculated with a-flagellar colonies of *L. adleri* grown in immune serum were similar to those in animals inoculated with active flagellates; cultures were obtained from the subcutaneous tissue four weeks after inoculation.

DISCUSSION

It is clear that *L. adleri*, a lizard Leishmania, produced transient infections with a duration of up to circ. five weeks in hamsters and in mice. In hamsters the infection is cryptic during the whole period of infection; in baby mice the infection was patent during a period of 10 days and subsequently became cryptic. The results in baby mice were similar to those produced by *L. enrietti*, a mammalian *Leishmania* which is not infective for hamsters. Mammalian Leishmanias which have ceased to be infective for adult laboratory animals after periods of passage in cultures (about 18 months in the case of *L. tropica*), or in some instances almost immediately after isolation, are often infective for baby mice. Infestation of connective tissue cells with L.D. bodies is typical of infections in these animals. It is noteworthy that in the above recorded experiments transient infections were obtained in adult hamsters by the inoculation of cultures passaged for almost three years on artificial media.

MOHUDDIN [8] has shown that *L. adleri* is infective for the following lizards: *Mabuia striata*, *Agama mutabilis*, *Acanthodactylus boshianus asper* and *Lacerta viridis*. In all these lizards the infection was cryptic and could be proved by culture of heart blood but not by direct microscopic examination. Lizards of the above species were negative after inoculation with *L. tropica* and *L. donovani*. ADLER & THEODOR [3] also obtained negative results after inoculating *Tarentola mauretanica* with *L. infantum*. It is thus evident that at least one species of lizard *Leishmania* is capable of infecting both reptilian and mammalian hosts although the infections in the latter were transient (up to five weeks duration) and cryptic except in baby mice where patency did not exceed ten days. It is not difficult to imagine that a *Leishmania* of this type became the ancestor of mammalian Leishmanias after having evolved, as a result of one or a number of mutations, from a stage of more or less prolonged survival to one of multiplication in a mammal into which it was introduced repeatedly by a sandfly vector.

It is not irrelevant to mention that Leishmanias of lizards restricted to the blood stream and internal organs have not as yet been found in America and a search in this direction would be of scientific interest.

RESUMO

Comportamento de leishmânia de lagartos em "hamsters" e camundongos recém-nascidos.

Leishmania adleri Heisch, uma leishmânia de lagartos do Quênia, parasito de *Latastia longicorda revoili*, produziu infecções crípticas transitórias em 6 dentre 24 adultos e 6 dentre 11 "hamsters" recém-nascidos inoculados intrasplênica ou então intraperitonealmente. A infecção durou até 5 semanas e sòmente pôde ser descoberta pela cultura de material do baço.

Dentre 71 camundongos recém-nascidos, inoculados por via intradérmica, 44 se infectaram. Em 19 a infecção era patente no tecido conjuntivo subcutâneo. Nenhuma infecção se patenteou após 10 dias da inoculação. Após êste período a infecção era críptica e podia ser demonstrada pela cultura de material do tecido subcutâneo.

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A *Leishmania adleri* tem antígenos em comum com tôdas as leishmânias humanas.

É discutida a relação entre leishmânias de mamíferos e de lagartos.

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