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Susceptibility of field, house and laboratory rodents to infection with Leishmania donovani.

CHARLES W. YOUNG and PAO-YUNG LIU.

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]*

After it has been demonstrated that the striped hamster *(Cricetulus griseus)* is easily infected with *Leishmania donovani*[1, 2] we desired to know how its susceptibility compares with that of the laboratory rodents previously used in the study of kala-azar. When the hypothesis of a rodent reservoir of kala-azar suggested itself, a study of the susceptibilities of the field and house rodents of an endemic area was indicated.

Rabbits and guinea pigs are known to be refractory to infection with *Leishmania.* White mice usually have been used; white rats less frequently and with less success. It was decided to study these two animals in order to compare them with the wild rodents. The forms investigated thus far are: (1) the giant hamster *(Cricetulus triton)*, (2) a vole *(Microtus* sp.*)*, (3) the Chinese house mouse *(Mus wagneri)*, (4) the house rat *(Mus rattus)*, (5) the white mouse *(Mus musculus albinus)*, a strain imported from America, and (6) the white rat *(Mus norvegicus (vel decumanus) albinus)*. The results of the study of this lot of white rats have been reported[3] but are repeated here for comparison with those of other rodents. So far as known, only the white rat and white mouse have been studied previously and there is lack of unanimity as to the susceptibility of the first of these.

The purpose of the present investigations was to determine whether the rodents named are susceptible to infection with *Leishmania donovani* and if so to what degree, and whether there is any tendency toward spontaneous recovery. Should such be

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1 Smyly, H. J., and Young, C. W., PROC. SOC. EXP. BIOL. AND MED., 1923-24, xxi, 354.

2 Young, C. W., Smyly, H. J., and Brown, C., PROC. SOC. EXP. BIOL. AND MED., 1923-24, xxi, 357.

3 Young, C. W., and Smyly, H. J. (in press).

found it was desirable to ascertain when recovery began and when it was complete.

Method. We have found that with our five laboratory strains of *L. donovani,* the liver and spleen from one positive hamster may be injected intraperitoneally into twenty new animals and that this dose will produce moderate to heavy positives in from one to two months. Doses for the rodents studied were made in proportion to the average weight of the species as compared with that of the striped hamster. Lots of a given species were inoculated at one time in order that each might receive the same number of organisms. This was necessary, because as yet there is no method of counting the non-flagellate form of *Leishmania.* It was planned to liver-puncture each animal ten days after inoculation in order to test whether it actually had become infected. This procedure had to be omitted in the case of some of the smaller species on account of the high mortality that resulted from the procedure. At ten-day intervals, estimated from the date of inoculation, two or three of the animals were killed with ether and autopsied aseptically. Smears and cultures (NNN medium) were made from the spleen, liver, bone marrow and heart blood. In some series, the peritoneal cavity was washed out with sterile Locke's solution and the washings centrifugalized. Smears and cultures were made from the sediment. Animals often died either as a result of liver puncture or from some intercurrent cause. Such carcasses were autopsied but as the organs became infected with bacteria soon after death, routine cultures were not made. In estimating the intensity of infection the following scale was used: + indicates less than an average of one *Leishmania* organism to each oil-immersion field (2.0 mm. objective; B and L 10X ocular); ++, between one and ten parasites; +++ between 10 and 100; ++++, between 100 and 500. The organisms were estimated approximately; not counted. Degree of susceptibility was taken to be indicated by the number of parasites per field in the smears. If none of a given species showed more than ++ or +++ we have said that such animals were only moderately susceptible to infection with *Leishmania donovani.* The criterion of "a tendency toward spontaneous recovery" was considered to be a decrease in the intensity of infection in the later members of a series.

Results. Elsewhere[2, 3] it has been shown that striped hamsters begin to show positive liver punctures before the tenth day after

inoculation, and that with rare exceptions there is no tendency toward spontaneous recovery.

Giant Hamster (two series of 19 and 15 hamsters respectively). Of 34 giant hamsters inoculated, 26 were positive at autopsy, four were negative and the organs of four were decomposed and therefore unreliable for diagnosis. The animals died or were killed at intervals of 25-320 days after inoculation. No tendency toward spontaneous recovery was noted. The organs of these animals frequently showed ++++ infections so that we consider them nearly or quite as susceptible as striped hamsters.

Vole (one series of 10 voles). On account of the difficulty in obtaining sufficient numbers of these animals and of keeping them alive in captivity, only one small series was studied. They showed infections of moderate intensity (++ or +++) after 13-21 days. No tendency toward recovery was seen in the individuals dying 37 and 38 days after inoculation.

House Rat (one series of 9 rats). This rodent is also difficult to maintain in captivity. None of the animals in the single series studied became heavily infected. Only one showed +++ in the spleen. Both rats that died 46 days after inoculation showed light (+) infections of the spleen and liver.

House Mouse (four series of 9, 17, 19, and 20 mice respectively). On the other hand, house mice are easily maintained and those dying 100-140 days after inoculation were moderately (++) to heavily (++++) positive. If there was any tendency toward spontaneous recovery, it was not marked.

White Rat (one series of 10 rats). In the one series studied, there were no infections above +, the smears became negative after 30 days and the cultures (except from the peritoneal cavity) also with the exception of that from the spleen of one 37 day animal.

White Mouse (two series of 15 and 18 mice respectively). These white mice became moderately infected (++, +++; rarely ++++) after about forty days. Animals killed after 70 days showed the same intensity. Two killed after 82 days were only + (one spleen smear ++) but no definite conclusion regarding tendency toward spontaneous recovery can be drawn from the small number of animals examined..

Discussion. None of the series of animals in which some members showed an apparent tendency to recover, was sufficiently prolonged to demonstrate undoubted spontaneous recovery. Such a tendency seemed to be present in house rats, white rats and to a less extent in white mice but the numbers of these rodents killed at each interval were too few to make the findings unequivocal. All the field rodents studied, as well as the house mice remained heavily infected throughout the period of observation

Conclusions. 1. The giant hamster *(Cricetulus triton)*, a vole *(Microtus* sp.) and the Chinese house mouse *(Mus wagneri)* are markedly susceptible to infection with *Leishmania donovani.* These showed no tendency toward spontaneous recovery during the periods covered by the present studies.

2. The house rat *(Mus rattus)* white rat *(Mus novegicus (vel decumanus) albinus)* and white mouse *(Mus musculus albinus)* show infections of only moderate intensity and seem to exhibit a tendency to recover spontaneously. However none of the series was long enough to show undoubted and complete recovery.

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A search for field and house rodents naturally infected with kala-azar.

CHARLES W. YOUNG and MARSHALL HERTIG.*

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

The striped hamster *(Cricetulus griseus)* has been shown to be very susceptible to infection with kala-azar.[1] Its southern range corresponds, so far as it has been studied, with the distribution of kala-azar in China. These two facts together with other obser-

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[1] Smyly, H. J., and Young, C. W., PROC. SOC. EXP. BIOL. AND MED., 1923-24, xxi, 357.

[2] Young, C. W., Smyly, H. J., and Brown, C., PROC. SOC. EXP. BIOL. AND MED., 1923-24, xxi, 357.