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Experimental kala-azar in a hamster.

(Cricetulus griseus, M.—Edw).

By CHARLES W. YOUNG, H. JOCELYN SMYLY and CABOT BROWN.

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

This paper is not intended to be an account of a completed problem. It is rather a progress report. On account, however, of the almost uniform success in infecting this hamster with kala-azar, it seems advisable to publish the data thus far obtained. Further search among related rodents may reveal a still more suitable animal.

The experimental study of kala-azar has been hampered by lack of susceptible laboratory animals. Those commonly used, such as the monkey, dog, white mouse and white rat are not uniformly infected even when the number of organisms injected is large. In 1919, Hsieh[1] introduced into use for pneumococcus grouping, a Chinese hamster *Cricetulus griseus,* M.-Edw. This rodent is found wild locally, and is easily and cheaply obtained. It thrives in captivity and is said to breed if earth is provided for burrowing. In another paper read at this meeting, we have described the experimental transmission of the North China kala-azar to animals. The first case (Hospital No. 3852) was a very heavily infected one. Among the animals inoculated were five hamsters, all of which became infected. The second (Hospital No. 4358) was a very lightly infected child. At autopsy the spleen and liver smears and cultures were negative. The marrow smears were positive. Of the hamsters inoculated and surviving the bacteria present in the material, all become infected with kala-azar, namely, one injected with spleen, two with liver and two with marrow. In the studies here reported the aflagellate form of *Leishmania donovani* or Leishman-Donovan body has been used. This was obtained, except the original inoculations, from mice or hamsters, usually the latter.

Experimental Work. Preparation of Material. The human material, spleen or liver was ground in a Rosenow tissue crusher,

[1] Hsieh, E. T., A New Laboratory Animal *(Cricetulus griseus). National Med. J. of China,* 1919, v, 20.

and suspended in 0.85 per cent saline or Locke's solution. In the case of that obtained from rodents, one liver and one spleen were ground as before and suspended, usually in 10 cc. of saline or Locke's solution and injected into 20 hamsters.

Intraperitoneal Inoculations. These were made with a sterile syringe carrying a No. 20 or 22 needle, both boiled in 0.85 per cent saline. The belly of the hamster was first sponged with alcohol. Examination as to whether the animal was infected, was made by liver puncture by a similar technique. In the latter case, gentle negative pressure was exerted by pulling on the piston of the syringe while withdrawing the needle. Intracellular organisms were found at varying intervals from 1 to 332 days after inoculation. The question arose in the earlier cases, whether the parasites found came from the liver indicating infection, or were from the unabsorbed inoculum. Twenty hamsters were injected each with 0.5 cc. of liver and spleen suspension from two other infected animals, and sacrificed four daily. In each animal, a study was made of smears from (1) centrifugalized peritoneal washings, (2) liver rinsed twice with saline to free it from the inoculum, (3) similarly washed spleen, and (4) bone marrow (femur). The intracellular forms persisted in the peritoneal cavity in large numbers throughout. Organisms appeared in the spleen (smears) first after 3 days, and in the liver and marrow after 4 days. The organisms in the liver were more numerous than in the marrow but the material used was much more suitable. In two other hamsters, 9 days after inoculation, intracellular parasites were found in the washings from the peritoneal cavity as well as in the washed liver and spleen smears. Peritoneal punctures were made on animals inoculated at varying periods from 28 to 334 days. The report of these will be given later. To summarize: 136 intraperitoneal inoculations have been made. One hundreds of these have been studied thus far. Omitting eleven negative cases of one, two and three day hamsters among the 20 noted above, the remaining 89 show the following results:

	Positive	Negative
Smears from organs	28	3
Liver punctures	51	5
Tissue sections	2	0
	81	8

This gives 91 per cent positive results from intraperitoneal inoculations.

Intrapleural Inoculations. Of two hamsters inoculated intrapleurally, one showed positive spleen smears fifteen days after inoculation. The second gave a positive liver puncture 53 days after injection.

Subcutaneous Inoculations. Eleven hamsters were inoculated subcutaneously. Of these four have been examined. One showed negative liver punctures after 22 and 28 days, but became positive after 53 days. The three others were autopsied 51 days after injection. One showed positive liver, spleen and marrow smears. The remaining two were negative.

Feeding Experiments. Five hamsters were starved 36 hours and then fed on the liver and spleen of an infected animal divided approximately equally among the five. Two died within eight hours and one other after seven days. No cause of death was found at autopsy. The two remaining animals were sacrificed 18 days after feeding. Liver and spleen smears were negative.

DISCUSSION.

The question of the earliest interval after intraperitoneal inoculation, at which liver puncture may be taken as a true criterion of systematic infection is still unanswered. While this is true it may be said that in no case in which early liver punctures were positive has the animal failed to show systemic infections at autopsy later. Our hamsters showed no tendency toward spontaneous recovery. The longer they lived the heavier the infection became. In one animal dying 325 days after infection (No. 7), the spleen weighed 1.52 grams, which is about 14 times the normal.

SUMMARY AND CONCLUSIONS.

A Chinese hamster (*Cricetulus griseus,* M.-Edw.) is very susceptible to inoculation with the aflagellate form of *Leishmania-donovani* when the organisms are injected intraperitoneally.

It is also susceptible to intrapleural inoculation.

It is sometimes but not uniformly infected by subcutaneous inoculation.

The two feeding experiments were negative.