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Splenectomy and Benzol Injection as Means of Increasing Susceptibility of Chinese Hamsters to Kala-Azar.

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It has been shown in experimental animals that splenectomy and benzol injection frequently give rise to an increase of susceptibility of the animals to bacterial, parasitic, as well as virus infections.[1, 2, 3] In parasitic infections such observations have been made with plasmodia, spirochetes and trypanosomes in monkeys, mice, rats, squirrels, etc. The effect of splenectomy and benzol injection on the susceptibility of the Chinese hamster, *Cricetulus griseus*, to *Leishmania donovani* has not been hitherto recorded. The following experiment was, therefore, designed, and the findings are here reported. The flagellate form of *Leishmania* was employed in the present study.

The experiment consisted of 3 groups of 20 hamsters each. The first group consisted of 20 splenectomized hamsters, the second, 20 benzol-injected hamsters and the third, 20 normal hamsters as controls. Splenectomy was carried out under ether anesthesia. All the splenectomized hamsters were allowed to recover from the effects of the operation for a period of from 1 to 2 months, before they were given the inoculation of flagellates. Of the 20 hamsters in the benzol group 10 were each injected subcutaneously with a single dose of 0.5 cc of a mixture of equal parts of benzol and sterile olive oil, 5 with 1 cc each, and another 5 with 2 cc each. The interval between the injection of benzol and inoculation of flagellates was 30 hours in the 0.5 cc group, 48 hours in the 1 cc group, and 7 days in the 2 cc group. From a preliminary determination it was found that the maximum tolerated single dose of the benzol mixture approaches closely to 2 cc for an average hamster. The inoculation of flagellates seems to be preferably given about 48 hours after the benzol injection. The flagellates used in the present study were prepared by pooling the condensation fluid of some 20 tubes of a 13-day culture which had been grown on NNN medium from the spleen emulsion of a heavily infected hamster. On May 10, 1939, each of the 60 hamsters in the 3 groups was given on the same day a single intraperi-

1 Adler, S., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1930, **24**, 75.

2 Meleney, H. E., *J. Exp. Med.*, 1928, **48**, 65.

3 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649.

toneal inoculation of 0.2 cc of the culture, estimated to contain 145,000 flagellates.[4]

After the inoculation of flagellates infection in the hamsters was first determined by liver puncture and then by the examination of smears and sections made from the spleen and liver at autopsy. Two liver punctures were done before the hamsters were sacrificed for examination. Only 3 or 4 hamsters from each group were punctured. The first puncture was done 29 days and the second, 56 days after the infective inoculation. The results of the first puncture were all negative. But by the time of the second puncture 1 or 2 hamsters from each group already showed positive smears. In order to make use of the rate of early infection to determine the degree of susceptibility, all the hamsters which had survived up to the time of the second puncture were sacrificed. Not only were smears and sections made from the spleen and liver of the hamsters but also the weight of the hamsters as well as that of their spleen and liver were recorded. During the period of infection 2 hamsters from the splenectomized group died. Their organs were not suitable for examination because of marked post-mortem changes. The results of the examination of the spleen and liver of all the remaining hamsters are shown in Table I in which only the parasitological findings of sections were recorded, as they were found to be more conclusive than those afforded by direct examination of smears.

As shown in Table I, in the group of 18 splenectomized hamsters which were killed and examined at the conclusion of the experiment all except one showed a large number of parasites in the sections made from the liver. On the other hand, in the group of control hamsters, only 8 out of 18 (liver of 2 hamsters not examined) hamsters gave positive liver sections, in most of which only a few parasites were found, although positive spleen sections were seen in 14 of the 20 hamsters. In this group 6 hamsters failed to take the infection. In the benzol-injected group 4 hamsters showed negative spleen sections and 9 showed negative liver sections. Four hamsters in this group failed to contract kala-azar. There was no striking difference of the infection rate among the various dosage groups of benzol-injected hamsters. The 2 cc group seemed to have given the highest infection rate, but repeated smaller doses which were not tried in the present study, might give a higher infection rate.

Table II shows the average weight of the hamsters and that of their spleen and liver. In the benzol group the weighing of the

4 Earle, W. C., and Perez, M., *J. Lab. and Clin. Med.*, 1932, **17**, 1124.

TABLE I.

Parasitological Findings of Sections of Spleen and Liver of Splenectomized, Benzol-injected and Normal Hamsters.

Splenectomized hamsters		Benzol-injected hamsters			Control hamsters		
Hamster No.	Leishman-Donovan bodies in liver sections	Hamster No.	Leishman-Donovan bodies in sections of liver	Leishman-Donovan bodies in sections of spleen	Hamster No.	Leishman-Donovan bodies in sections of liver	Leishman-Donovan bodies in sections of spleen
3003	++	3035	+++	+++	3045	(+)	+++
3004	+	3036	++	+++	3046	0	++
3005	died (not examined)	3037	+	+++	3047	++	+++
3006	" " "	3038	+++	+++	3048	0	(+)
3007	++	3039	+++	+++	3049	0	0
3008	(+)	3040	0	+	3050	0	0
3009	+++	3041	0	(+)	3051	+++	+++
3010	0	3042	0	0	3052	0	0
3011	++	3043	0	0	3053	+	+++
3012	++	3044	(+)	+++	3054	0	0
3013	+++	3070	0	0	3082	0	++
3014	++	3071	(+)	++	3083	0	0
3015	++	3072	+	+++	3084	(+)	++
3016	+++	3073	0	0	3085	(+)	++
3017	+++	3074	+	+++	3086	—	++
3018	+++	3065	0	+	3087	—	+++
3019	+	3066	+	+++	3088	(+)	++
3020	+++	3067	0	++	3089	0	0
3021	++	3068	++	++	3090	(+)	(+)
3022	+++	3069	0	++	3091	0	(+)

Hamsters Nos. 3035-3044 incl. each received 0.5 cc of benzol mixture.
" " 3070-3074 " " " 1.0 " " " "
" " 3065-3069 " " " 2.0 " " " "

— = Not examined.
0 = Not found.
(+) = Very few found.
\+ = Found in every 11-20 oil immersion fields.
++ = " " " 6-10 " " "
+++ = " " " 1-5 " " "

TABLE II.
Average Weight of Body, Spleen and Liver of Splenectomized, Benzol-injected and Normal Hamsters.

	Body weight, g	Liver weight, g	Spleen weight, g
Control hamsters	26.1	1.023	0.089
Splenectomized hamsters	28.7	1.320	—
Benzol-injected hamsters	26.6	—	0.105

liver was not done. Taking the normal weight of the hamster's spleen as from 0.15 to 0.3% of the body weight[5] it is evident that the average weight of the spleen in both benzol-injected and control hamsters was above normal. The increase of the spleen weight was apparently due to the kala-azar infection. The difference of the average spleen weight between these two groups seems to be out of proportion to the difference of their average body weight. As the rate of spleen infection was higher in the benzol-injected hamsters than in the controls, it appears likely that the greater average spleen weight of the benzol-injected group was due to the heavier infection rather than the greater average body weight. In the splenectomized hamsters the average liver weight was again greater than that of the controls. This was also likely due to the heavier infection rather than the greater average body weight of the splenectomized animals, although a part of the increased liver weight might have been due to a compensatory hyperplasia of the reticulo-endothelial system.

Conclusions. Chinese hamsters became more susceptible to infection with the flagellates of *Leishmania donovani* after splenectomy or benzol injection Of the two means employed to increase the susceptibility of hamsters to kala-azar infection splenectomy appears to be more effective.

[5] Meleney, H. E., *Am. J. Path.*, 1925, **1**, 147.