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Neostibosan and Experimental Kala-Azar in Chinese Hamsters. I. Normal Hamsters.

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The problem of chemotherapy of experimental kala-azar was investigated by Smyly,[1] who used 4 drugs, to treat infected Chinese hamsters, *Cricetulus griseus*. None of Smyly's infected animals was cured of the infection, although sodium antimonyl tartrate was given in large doses for a period sufficient to effect a cure in man. Hindle, working with Johnson,[2] obtained similar negative results with neostam, novostiburea, antimosan, and antimonyl tartrate, all of which have been successfully used in the treatment of kala-azar in man. Roehl[3] studied the action of tartar emetic, antimosan, stibosan, and neostibosan, in infected European hamsters, *Cricetulus frumentarius,* and was able to keep his treated animals free of parasites for months. Recently, Kikuth and Schmidt[4] obtained similar positive results with solustibosan. The contradictory results of Smyly and Hindle on one hand and Roehl and Kikuth and Schmidt on the other, in the opinion of the last two workers, are due to the different species of animals used. Whether Chinese hamsters, infected with *Leishmania donovani* can be cured of the infection, and whether the difference in species explains the difference in results, are the problems under investigation. Neostibosan was used because it possesses high curative value in human kala-azar and can be given subcutaneously.

Experiment 1. Determination of the *maximum non-lethal dose, maximum tolerated dose,* and *universal lethal dose* of neostibosan for hamsters. By *maximum non-lethal dose* we mean the amount given as a single injection, without killing any of the animals; *maximum tolerated dose* is the amount given as a single injection, which kills most of the animals; *universal lethal dose* is the amount given as a single injection, which kills all the animals. One hundred normal hamsters were used. Seventy-two percent of these animals weighed 30 g each; the rest varied from 25 to 36 g. The animals were divided into 5 groups, each con-

1 Smyly, H. J., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1926, **20**, 104.
2 Quoted from 4.
3 Roehl, W., *Ind. Med. Gaz.*, 1929, **64**, 563.
4 Kikuth, W., and Schmidt, H., *Chinese Med. J.*, 1937, **52**, 425.

sisting of 10 males and 10 females. Each group received a single injection of one test dose. Five test doses, 0.8 g, 1.0 g, 2.0 g, 3.0 g, and 4.0 g per kg were used. Neostibosan was freshly prepared each time in a 5% sterile aqueous solution, and given subcutaneously in abdominal wall of the animal by means of a tuberculin syringe. Any death within 48 hours after injection was considered as due to the drug. This arbitrary time limit was chosen because the excretion of pentavalent compounds of antimony is approximately 50% in the first 24 hours.[5, 6, 7] Any death occurring later than 2 days after injection is not likely to be due to the direct effect of antimony. The results of the 5 test doses are given in Table I.

TABLE I.
Mortality Rate of Hamsters to Test Doses of Neostibosan.

Group	Dose, g per kilo	No. of animals	No. of deaths	Mortality %
A	0.8	20	0	0
B	1.0	20	0	0
C	2.0	20	5	25
D	3.0	20	13	65
E	4.0	20	20	100

Among the 5 animals in Group C which died there were 2 males and 3 females, each weighing 30 g. Among the 13 deaths in Group D there were 6 males and 7 females (11 weighing 30 g each, and 2 weighing 29 g each). It seems that males are just as susceptible to the drug as females. From the table it is evident that among the doses tried, 1.0 g per kilo is the maximum non-lethal dose, 3.0 g per kilo, the *maximum tolerated dose,* and 4.0 g per kilo, the *universal lethal dose* of neostibosan for Chinese hamsters.

Experiment 2. Relation of body weight to the *maximum non-lethal dose.* Thirty hamsters were used. The results are shown in Table II.

TABLE II.
Relation of Body Weight of Hamsters to Maximum Non-lethal Dose of Neostibosan.

Body wt. (g)	11	12	14	15	16	17	20	24	29	30	32	34	37	40
No. of animals	1	4	2	2	2	5	3	2	1	2	2	2	1	1
No. of deaths	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Except for one death among the 3 animals which weighed 20 g each, the *maximum non-lethal dose* is universal to animals weighing

[5] Weese, H., *Chinese Med. J.*, 1937, **52**, 421.

[6] Boyd, T. C., and Roy, A. C., *Ind. J. Med. Res.*, 1929, **17**, 94.

[7] Brahmachari, U. N., Chondbury, S. C., Das, J., and Sen, P., *Ind. J. Med. Res.*, 1924, **11**, 829.

TABLE III.
Cumulative Effect of Repeated Doses of Neostibosan.

Dose (mg/kilo)	200	300	400	500	600	700	800	900	1000	1200	1400	1600	1800	2000
No. of animals	20	20	20	19	19	19	19	17	17	17	15	10	5	2
No. of deaths	0	0	1	0	0	0	2	0	0	2	5	5	3	2
Mortality %	0	0	5	0	0	0	10.5	0	0	11.7	33.3	50	60	100
Cumulative mortality	0	0	5	5	5	5	15	15	15	25	50	75	90	100

TABLE IV.
Effect of a Second Injection of Maximum Non-lethal or Toxic Dose of Neostibosan.

Dose (g per kilo)	First injection			Second injection			Interval between injections in days
	No. of animals	No. of deaths	Mortality %	No. of animals	No. of deaths	Mortality %	
1	20	0	0	20	5	25	34-50
2	20	5	25	9	4	44.5	34-51
3	20	13	65	5*	5	100	34-65

*Two animals only received 2.0 g per kilo in the first injection.

from 11 g to 40 g. The single death out of 30 hamsters may be considered as an accident. There seems to be no difference, therefore, in the susceptibility of hamsters of different body weights to the *maximum non-lethal dose* as previously determined.

Experiment 3. Cumulative effect of repeated injections and the determination of the *probable therapeutic dose.* Since repeated injections of neostibosan are required to effect a cure of kala-azar, one must know whether there is increased tolerance or cumulative effect, and find out a dose suitable for repeated injection at certain intervals with least toxic effect, to the animal.

Ten males and 10 females, each weighing 20 to 27 g were used. Injections of 1% solution of neostibosan were given subcutaneously, twice a week. The doses began at 200 mg per kilo, and were increased by 100 mg steps up to 1 g per kilo, and then by 200 mg steps up to 2 g per kilo. The results are given in Table III. The mortality rate following a single injection of 2.0 g neostibosan per kilo body weight was 25%, but after injections of increasing doses given twice weekly of doses increasing up to a final dose of 2.0 g per kilo, the mortality rate increased to 100%. This strongly speaks for a cumulative effect, and against increased tolerance. One hamster out of 20 was killed when the dose reached 700 mg per kilo. For therapeutic use in infected hamsters, it would seem, therefore, that a constant dose of 400 to 500 mg per kilo body weight, given twice a week, might be regarded as the *probable therapeutic dose* of neostibosan, but various possible influences of the infected state of hamsters with kala-azar require investigation.

Experiment 4. Effect of a second injection of *maximum non-lethal dose,* and *toxic dose.* Any dose greater than the *maximum non-lethal dose* must be regarded as a *toxic dose.* The following experiment is to observe the effects of a second injection of the same *maximum non-lethal dose* and of *toxic doses* in animals which have survived a previous injection. The interval between the injections varied from 34 to 65 days. The number of animals used for the second injection was necessarily smaller than that for the first as many died of the first dose and some were otherwise lost. The results are shown in Table IV. There was a higher mortality following the second injection, even after one or 2 months, during which the animals were expected to have recovered from the previous dose. This decreased tolerance may mean that the first dose though not fatal, damaged the organism in some way so as to lower its tolerance to the subsequent injection.

Summary and Conclusions. 1. In normal Chinese hamsters it was

found that the *maximum non-lethal dose* of neostibosan is 1.0 g per kilo body weight; the *maximum tolerated dose,* 3.0 g per kilo body weight; and the *universal lethal dose,* 4.0 g per kilo body weight. 2. The sex and body weight of hamsters played no significant rôle. The *maximum non-lethal dose* was found universal to animals weighing from 11 to 40 g. 3. There was no increase of tolerance after repeated injections of gradually increasing doses of neostibosan. A cumulative effect was clearly shown. A *probable therapeutic dose* was found to be about 0.4 g per kilo body weight. 4. After an animal had been poisoned by a large dose of neostibosan, the repetition of the same or greater amount even after an interval as long as 2 months usually resulted in death.

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Neostibosan and Experimental Kala-Azar in Chinese Hamsters. II. Infected Hamsters.

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This report deals with observations on the effects of neostibosan on kala-azar in Chinese hamsters. As this work was started before Part I was completed, the *probable therapeutic dose* of neostibosan suggested in that communication was not used.

Twenty-six normal and 26 infected hamsters were equally divided into 4 groups, each consisting of 13 hamsters. Groups A and C were given repeated injections of neostibosan, while Groups B and D were kept as controls. Treatment was started 47 days after the infection with kala-azar. One percent sterile aqueous solution of neostibosan was given twice a week by means of a tuberculin syringe, subcutaneously in 7 hamsters and intramuscularly in 6 hamsters of each group. Hamsters were weighed weekly or fortnightly and the amount of neostibosan was given according to the latest weight. The first dose was 8 mg per kilo, which was continued for 6 weeks; this amount was chosen because it is effective in human kala-azar. As soon as this dose was found to be ineffective and the *maximum non-lethal* dose was found to be 1 g per kilo, the amount was raised to 200 mg per kilo and gradually to 800 mg. Altogether 32 injections, a total of 7 g per kilo, were given to each hamster in a period of 114 days.