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.

9979

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

C. W. WANG AND C. U. LEE. (Introduced by F. R. Dieuaide.)
From the Department of Medicine, Peiping Union Medical College, Peiping, China.

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.

Difference in tolerance of normal and kala-azar hamsters. The results are shown in Table I. The mortality rate is higher in Group A than in Group C which seems to indicate that tolerance for neostibosan is less in infected than in normal hamsters, but the difference is not statistically significant. No difference in mortality rate was seen between subcutaneous and intramuscular routes of administration.

TABLE I.
Tolerance of Normal and Kala-azar Hamsters for Neostibosan.

Group of animals				No. of animals	No. of deaths	Mortality %
A.	Infected hamsters	receiving	drug	13	7	53.8
B.	"	"	no drug	13	3	23.0
C.	Normal	"	drug	13	4	30.7
D.	"	"	no drug	13	2	15.4

Curative value of neostibosan in hamsters infected with kala-azar. The 32 injections of neostibosan given to Group A constituted a course of treatment. Group B served as controls. During the treatment several infected hamsters died. At autopsy the size of spleen and number of parasites were found to be decreasing with the increasing intensity of treatment. When a total dose of 2.3 g per kilo was reached, parasites were absent in almost all cases in smears made from the liver pulp obtained by puncture, while numerous parasites were found in smears of splenic pulp obtained by puncture. When a total of 7 g per kilo was reached, none of the 6 surviving hamsters showed parasites in smears of splenic pulp. A further splenic puncture was done 3 weeks after completion of the treatment in 3 of the animals and none of the smears showed parasites. Without further study one would consider these 6 hamsters apparently cured.

On completion of the treatment of kala-azar hamsters in Group A, 3 out of 13 controls in Group B had died. At autopsy all were found to have big spleens and leishmania were seen in smears. Liver puncture was done on each of the 10 survivors, and all but one were found to be heavily infected. In the negative case, the animal was later sacrificed. Its spleen and liver were found to be normal in size and no leishmania could be found in smears from these organs. It appears that this animal spontaneously recovered within about 5 months after infection, although infected hamsters usually show no such tendency.[1, 2]

[1] Smyly, H. J., and Young, C. W., PROC. SOC. EXP. BIOL. AND MED., 1924, **21**, 354.

[2] Young, C. W., Smyly, H. J., and Brown, C., *Am. J. Hyg.*, 1926, **6**, 254.

TABLE II.
Amount of Neostibosan Received by Apparently Cured Hamsters.

Hamster No.	1029	1032	1035	1036	1041	1044
Avg wt of animal, g	26	24	20	25	24	22
Total dose, mg	192.8	174.8	149.8	185.3	181.9	155.7
" " g per kilo	7.4	7.3	7.4	7.4	7.6	7.0

Further study was made of these apparently cured hamsters (Table II), except for one (1036) which was too decomposed for use. Hamsters 1029, 1032, and 1041 were sacrificed 10 days, 3 weeks, and 3 months respectively, after completion of treatment. The spleen of each of these hamsters was ground in 3 cc normal saline solution and 0.5 cc of this emulsion intraperitoneally inoculated into 6 normal hamsters. The spleens were all small and smears made from the emulsions were all negative. Two to 4 months later the normal hamsters which had been inoculated with the emulsions from hamsters 1032 and 1041 came down with kala-azar, proving that these 2 apparently cured hamsters were not actually cured. The normal hamsters which had been inoculated with the spleen emulsion of hamster 1029 never contracted kala-azar, proving that this apparently cured hamster was actually cured.

The other 2 apparently cured hamsters, 1035 and 1044, were reinoculated 2 to 4 months after completion of treatment with 0.2 cc splenic emulsion containing numerous *Leishmania donovani.* Two months later they were killed. Hamster 1035 was found to have enlarged spleen, with parasites in spleen and liver, while hamster 1044 was found to have normal spleen and liver, with no parasites in these organs. This showed that one was not reinfectable, while the other had either been reinfected or possibly suffered a relapse. It is noteworthy that only one (1029) of the 5 apparently cured hamsters was proved to be actually cured; while a second (1044) may have been cured. No difference was seen in the curative effect of neostibosan between subcutaneous and intramuscular routes.

Further observations on the curative value of neostibosan in hamsters infected with kala-azar. A new lot of 25 hamsters which had been infected with *Leishmania donovani* for 43 days were given biweekly subcutaneous injections of 1% solution of neostibosan. Nine injections were given, beginning with a dose of 200 mg per kilo, and gradually increasing to 900 mg per kilo. Four hamsters died during the course of treatment, while 21 survived the treatment, each receiving a total of about 5.0 g per kilo. All of these were apparently cured (negative liver and spleen punctures). Three of the apparently cured hamsters were sacrificed 10 days, 18 days, and 3 months

respectively after completion of treatment. All the smears made directly from the liver and spleen were negative for parasites. The spleen of each of these hamsters was used for hamster inoculation. The inoculated test hamsters were sacrificed 2 to 5 months later and the results showed that 2 of the 3 apparently cured hamsters were actually cured, but not the third whose splenic emulsion, though showing no parasites on direct study, was still capable of causing kala-azar. Twelve of the apparently cured hamsters were subjected to reinoculation 3 months after completion of treatment. About 2½ months after reinoculation one of the 7 hamsters which had received 0.1 cc of the infective material, showed evidence of kala-azar, but the 6 others were free from the infection. On the other hand, 3 out of the 5 hamsters which had received 0.2 cc of the infective material, showed evidence of kala-azar which, however, failed to develop in the others. In all, 4 or 33.3% of the 12 apparently cured hamsters were reinfected, or possibly had a relapse, while 8 or 66.7% were refractory to reinfection. The reinfectability of the apparently cured hamsters varied with the reinfective dose. Among 20 normal hamsters which had been inoculated with the same infective material as that given to the 2 apparently cured hamsters in the preceding experiment and 12 in this experiment, all except one developed kala-azar at end of 2½ months. Six of the apparently cured hamsters were kept under observation for 5 months after completion of treatment and then sacrificed. Four of the 6 were found to have enlarged spleens and livers, all harboring *Leishmania donovani,* a relapse rate of 66.7%. The 2 others had normal-sized spleens and livers with no parasites in these organs, and were apparently cured (Table III).

TABLE III.
Curative Effect of Neostibosan in 26 Apparently Cured Hamsters.

Nature of test	Result	No. of animals
Hamster inoculation test	Cured	3
	Not cured	3
Reinoculation test	Reinfectable	5
	Not reinfectable	9
Observation for relapse	Relapse	4
	No relapse	2

Discussion. There were altogether 26 apparently cured hamsters among the 38 which were systematically treated. Of these apparently cured hamsters 6 were tested by hamster inoculation and 3 were found to be actually cured, giving a cure-rate of 50% among the hamsters which survived the treatment. It is obvious that for

experimental purposes the most reliable criterion of cure is inoculation of a splenic emulsion into normal hamsters. The 12 hamsters which died during the course of treatment indicate a high mortality, 31.6%. As the natural death-rate of hamsters, either non-infected or infected (Table I) is considerable, it is difficult to say how far this mortality was directly attributable to neostibosan.

The range of the effective dose of neostibosan for hamsters seems to vary from 200 to 500 mg per kilo. This dose agrees well with the *probable therapeutic dose* of 400 mg per kilo proposed in Part I. The total amount of neostibosan per kilo body weight used to effect cure in a hamster is about 100 times the human dose. In view of this, the failure of Smyly and Hindle to obtain cures in their experiments was probably due to inadequate dosage and insufficient treatment, rather than to the difference in species of hamster. Even with this enormous dose the cure rate was only 50% among the hamsters which survived the treatment and were tested by hamster inoculation, much lower than the cure-rate in man. Whether or not a 100% cure rate can be achieved in experimental kala-azar is a problem for further investigation.

That 9 of the 14 hamsters apparently cured of kala-azar by neostibosan (Table III) were refractory to a second inoculation with *Leishmania donovani* is suggestive of immunity, but one can not be sure that a focus of infection was not present. The relapse rate of 66.7% is considerable, and further suggests that the treatment given was insufficient. The occurrence of spontaneous recovery is interesting, but rather infrequent. On the whole it seems that kala-azar is more difficult to treat in hamsters than in man.

Summary. Chinese hamsters infected with kala-azar seemed to have a lower tolerance for neostibosan than normal hamsters, but the difference was not statistically significant. Five to 7 g of neostibosan per kilo body-weight constituted a course of treatment. The range of the effective dose was from 200 to 500 mg per kilo. The mortality rate during treatment was 31.6%. The apparent cure-rate in hamsters surviving the treatment was 100%, but the actual cure-rate was only 50%. An inoculation test is necessary to prove actual cure. Nine out of 14 apparently cured hamsters were refractory to a second inoculation with *Leishmania donovani*. Spontaneous recovery from and relapse of kala-azar were seen in Chinese hamsters.