

The principal feature is the division of the total volume into 2 or more portions. The burette shown in the accompanying figure has a capacity of 50 cc. and is divided into 3 arms. Arm A has a capacity of 2 cc., or preferably a little more, and is graduated to 0.01 cc. Arm B consists of ten 2-cc. bulbs, while Arm C consists of three 10-cc. bulbs. These bulbs are conveniently blown out of a 2 mm. capillary tubing. The 3 arms are joined together above the graduations by a 2-mm. capillary tubing. The volume of this connecting piece need not exceed 0.1 cc. The constrictions in Arms B and C have the same internal diameter as Arm A. The lower ends of the arms, provided with cocks, are joined together and connected to a manometer and a levelling bulb.

For a 10 cc. burette 2 arms will suffice. One arm consists of nine 1-cc. bulbs while the other arm is a 1 cc. burette graduated to 0.01 cc.

With Arms A and B filled with mercury to the zero mark, cocks a and b closed, and c open, the gas to be measured is introduced into the burette. If the volume of the gas, estimated in Arm C is, for example, between 20 and 30 cc., the mercury in C is brought to the 20 cc. mark, cock c is closed and cock b is opened. If the reading in B is between 8 and 10 cc., the mercury is brought to the 8 cc. mark, b is closed and a is opened. The final reading of the volume is taken on arm A. The total volume of the gas measured is, of course, the sum of the volumes in the 3 arms plus that of the connecting piece above the graduations.

5245

Transmission of Kala-Azar to Hamsters (*Cricetulus griseus*) by the Oral Route.

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Prior to 1928, probably only 4 flawless successful experimental transmissions of kala-azar *per os* were recorded in literature. Monkeys were employed by Archibald,¹ who used infected human material, and also by Greig and Christophers,² who injected by means of a hypodermic syringe human material obtained by splenic puncture and cultures of *Leishmania donovani* into the jejunum. Tran-

¹ Archibald, A. C., *J. R. A. M. C.*, 1914, **23**, 479.

² Greig, E. D. W., and Christophers, S. R., *Ind. J. Med. Res.*, 1916, **13**, 151.

sient infection was obtained by Cornwall and La Frenais,³ who fed bread soaked in cultures of *Leishmania donovani* to a white rat. Cultures also produced infection when fed to a white mouse, the subject of experiment by Christophers, Shortt and Barraud.⁴

Using Chinese hamsters (*Cricetulus griseus*), Shortt, Craighead and their colleagues reported^{5, 6} 9 successes by feeding emulsion of infected organs. Their culture experiments were also positive. Young, Smyly and Brown,⁷ however, failed twice to produce infection in the same rodents by feeding infected spleen and liver. Young, Hertig and Liu⁸ did not fare better when they fed whole carcasses of hamsters infected with kala-azar to normal ones during a period of 47 days. It is with the object of checking the conflicting results of these workers that the present investigation was undertaken.

After 12 hours' fasting, 30 normal hamsters were each fed by means of a pipette 0.5 cc. of a thick saline emulsion prepared from the spleens and livers of 4 hamsters heavily infected with kala-azar. This finely ground emulsion, swarming with *Leishmania donovani* was readily swallowed by the hungry animals.

Four (F-1 to F-4) of them were killed at half hourly intervals after feeding, another lot of 4 (F-5 to F-8) at 1, 2, 2, 3 hour periods, 7 (F-9 to F-15) after one day, 6 (F-16 to F-22) at weekly intervals and the remaining 9 animals allowed to die naturally.

Examination of gastric and intestinal contents was discontinued after the 7th day but heart's blood, spleen, liver, abdominal and cervical glands, bone-marrow, testes, kidney, and brain were examined during the entire experiment for Leishman-donovan bodies both by staining and culture. The gastric and intestinal contents of 12 (F-1 to F-12) hamsters killed during the first 4 days after feeding were, in addition, examined immediately after death. The results of these studies are summarized in Table 1.

Leishman-donovan bodies in flagellate forms were for the first time detected on the 37th day in a culture of spleen of a hamster killed 7 days after the feeding. Previous to this, culture and smears from all hamsters killed during the first 7 days after feeding (F-1 to

³ Cornwall, J. W., and La Frenais, H. M., *Ind. J. Med. Res.*, 1916, **3**, 698.

⁴ Christophers, S. R., Shortt, H. E., and Barraud, P. J., *Ind. Med. Res. Memoir*, 1926, **4**, 89.

⁵ Shortt, H. E., Craighead, A. C., Smith, R. O. A., and Swaminath, C. S., *Ind. J. Med. Res.*, 1928, **16**, 271.

⁶ Shortt, H. E., Craighead, A. C., and Swaminath, C. S., *Ind. J. Med. Res.*, 1929, **17**, 335.

⁷ Young, C. W., Smyly, H. J., and Brown, C., *Am. J. Hyg.*, 1926, **6**, 254.

⁸ Young, C. W., Hertig, M., and Liu, P. Y., *Am. J. Hyg.*, 1929, **10**, 183.

TABLE I.

Hamster No.	Days after feeding	Splenic enlargement	Organs positive for L. D. bodies		Remarks
			Stained smears	Cultures	
F-1 to F-14	½ hr. to 6 days	0	0	0	Cultures kept incubating for 46 to 51 days
F-15	7	0	0	Spleen on 37th day	Other organs neg. Cultures kept for 48 days
F-16	12	+	0	Spleen on 14th day	Ditto
F-17	19	0	0	Spleen (25th day) Blood (36th day)	"
F-18	26	+	Spleen	0	Cultures kept for 53 days
F-19	34	+	Liver, bone-marrow	Spleen (10th day)	Liver culture contaminated
F-20	45	+	Spleen	Spleen (15th day)	Cultures kept for 34 days
F-22	51	+	Spleen, liver, m.g. bone-marrow, kidney	Spleen, blood, mesenteric glands	
F-23	11 mos.	0	Mesenteric glands, kidney	Cultures contaminated (F-23)	
F-24	142	?	Spleen, liver	Joint (12th day)	Other cultures contaminated
F-25	143	+	Spleen, liver, bone-marrow, blood, m. glands, brain, spinal cord	Brain (21st day) testis (9th day)	Ditto
F-26	131	+	Spleen, liver, bone-marrow, blood, kidney, brain, joint	Cultures contaminated	"
F-27	177	+	Ditto (in addition, testes)	Spleen	"
F-28	95	+	Spl., liver, b.m., blood, brain	Spleen, liver, kidney	
F-21	7	0	0	0	Cultures kept for 72 days
F-29	Escaped				
F-30	319	Slight	0	?	

F-14 and F-21) showed no parasites, though the cultures from their organs were incubated from 46 to 51 days, during which time 3 examinations of each culture were made. Twelve hamsters killed or dying from 7 to 319 days after being fed with parasites showed *Leishmânia donovani* either in smears or cultures or both. Animals dying naturally showed enormous splenic enlargement. One hamster (F-23) was too decomposed for examination. Another (F-29) escaped. The last (F-30), sacrificed (Oct. 29, 1930) after 319 days, had a moderately enlarged spleen but the smears were negative for parasites; the result of the cultures is yet unknown.

From examinations of stomach and intestinal contents (fresh and stained), it was noted that though liver tissue was distinguishable up to 24 hours, Leishman-donovan bodies, even one hour after ingestion, could be made out with difficulty. A few degenerated ghosts of parasites were seen in an eighth hour hamster. No flagellate forms were observed.

The cervical glands of 7 hamsters (F-8 to F-14) under observation from the tenth hour to the sixth day were found to be of normal size and free from parasites, indicating that no injury to the oral mucosa was inflicted during the feeding process. The mesenteric glands were negative up to the fifty-first day although the infection of bone-marrow and kidney at this time shows generalized parasitization.

Conclusion. Excluding the 14 hamsters sacrificed within the first week, F-23 which was too much decomposed for examination and F-29 which escaped, the *per os* experiment registers an infection of 12 out of 14 hamsters—a result comparable to that (9 out of 11) obtained by the Indian workers. Young and his associates in Peiping, working with the same parasites used in these experiments, may have failed to produce infection *per os* because they did not grind and dilute the organs used, thereby facilitating phagocytosis and absorption; furthermore, they made no cultures of the organs of animals fed with *Leishmania donovani*.

In these experiments, evidence of infection became apparent on the 7th day after feeding parasites and generalized parasitization was found on the 51st day. The majority (5 out of 7) died within the period of 100 to 200 days, the course of the infection being about the same as that after infection by the intraperitoneal route with very dilute emulsion of kala-azar organs.

No flagellate forms were noted in the contents of the gastrointestinal tract in an observation on 12 hamsters extended from half an hour to 4 days. This is to be expected as the medium was contaminated and temperature too high for transformation into flagellates.