out on the third, fourth, and fifth generations. One group of the animals of the third generation, which did not receive any addition of vitamin C, was used as controls.

The experiment has extended almost 20 months. The basal diet was composed of: Soybean meal 35, mung bean meal 30, millet flour 30, NaCl 1, $CaCO_3$ 1.5 and cod liver oil 2. These ingredients, mixed with boiling water, were cooked in a double boiler for 20 to 30 min. This diet invariably produced scurvy in guinea pigs and caused death in less than 4 weeks. As the source of vitamin C fresh cabbage (Peking variety) was used in amounts of 2 gm. per rat each day. The same amount fed to guinea pigs prevented scurvy indefinitely and supported normal growth.

At the age of 8 months the third generation rats on cabbage feeding gave the following body weights in gm.: 282, 334, 360, 340 (males), 264, 272, 248, 230 and 290 (females); those on basal diet alone: 258, 346, 330, 322 (males), 262, 216, 244, 218 and 230 (females). The cabbage ration rats successfully reared 3 out of 4 litters; those on the basal diet, 2 out of 4 litters. The 4 animals of the fourth generation on cabbage feeding at the age of 18 weeks weighed 192, 188 gm. (males), 170 and 164 gm. (females). Two litters were cast but not reared. The 4 rats of the fourth generation on basal diet at the age of 17 weeks and 5 days gave the following body weights in gm.: 206, 254 (males), 158 and 172 (females). One small litter constituting the fifth generation was successfully weaned at the age of 23 days.

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New Technique for Feeding Sandflies (Phlebotomus) for Experimental Transmission of Kala-Azar.

C. H. HU AND C. U. LEE. (Introduced by Hsien Wu.)

From the Departments of Pathology and Medicine, Peking Union Medical College.

In the experimental attempts to transmit kala-azar by the bites of sandflies, it has been found desirable to substitute an artificial feeding on blood rich in Leishmann-Donovan bodies for the natural feeding upon infected animals, in order to obtain an abundant growth of flagellates within the flies. This blood is obtained by mixing the blood of a normal animal with the crushed spleen of a hamster heavily infected with kala-azar.

For such artificial feeding we used the technique of Hertig and Hertig.[1] This technique was learned in a few days and in the course of 2 months during the summer of 1928, 565 flies were fed. During this work it became clear that this technique had a number of limitations. The flies had to be fed one by one, making the procedure tedious. During the etherization and handling of the flies, many were injured. A satisfactory engorgement was obtained with *P. sergenti,* while *P. major* only in rare instances could be fully engorged. In order to feed a larger number of flies with a minimum of injury and a maximum of engorgement, the following technique has been devised.

Technique. Etherize a hamster heavily infected with kala-azar, pin its feet on a wooden board, and place its head and neck between the arms of a U-shaped wooden block. Remove the hair over the anterior surface of the neck and chest by pulling and sterilize the skin with alcohol and ether. With aseptic precautions, make a median incision through the skin extending from the anterior end of the lower jaw to the middle of the sternum. Pull apart the skin flaps and pin them to the arms of the U-shaped block, the upper surface of which is slightly raised above the level of the head and neck. Cut the carotid artery on one side and, as the blood wells up, quickly draw it into a sterile pipette. Practically all the blood that is obtained by bleeding can be drawn into the pipette. Defibrinate and centrifuge the blood.

Remove the spleen aseptically, cut the whole or a part of it in small pieces, and place it in a small test-tube 3 inches long and 1/3 inch in diameter. Crush it thoroughly with a glass rod. Into this crushed material stir the blood plasma and centrifuge the mixture to throw the cell clumps to the bottom. Add enough red cells to the supernatant fluid to give the resultant mixture the approximate concentration of normal blood.

Place a piece of flannel in the shallow dish on the upper surface of an aluminum feeding stage (Figs. 1 and 2B) and add the blood-parasite mixture. In order thoroughly to saturate the flannel piece, work the mixture into the flannel by repeated pressing. This flannel represents the subcutaneous tissue of the animal. Cover with a piece of skin from the hamster, removed during the centrifuging of the spleen-plasma mixture. The skin most suitable for this purpose is that of the back. Before applying the skin to the stage, remove the hair on the external surface and also the thin layer of muscle attached to the inside. Do not sterilize the external skin surface, as the flies do not bite as well on sterilized skin. Place the

[1] Hertig, Arthur T., and Hertig, Marshall, 1927, lxv, 328.

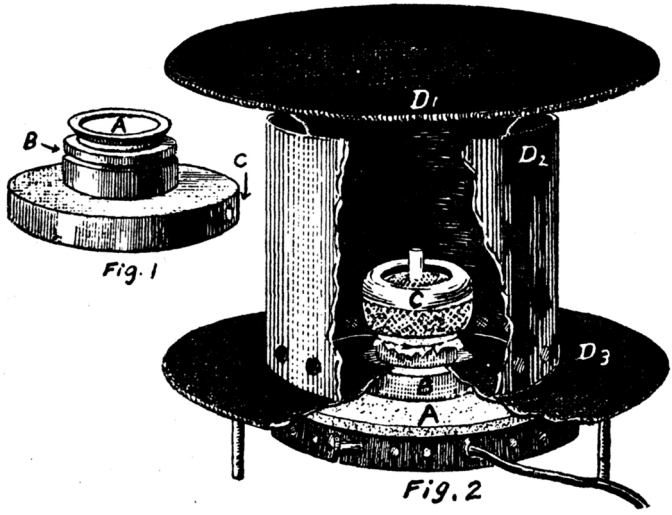

FIG. 1. *Feeding Stage.*

(A) Dish, about ¾ inch in diameter, for the holding of flannel. (B) the groove for the tying of the skin which covers the flannel. (C) a hole for the insertion of a thermometer to register the temperature of the feeding stage.

FIG. 2. *Feeding Outfit.*

(A) The electric heater (this heater is manufactured by Chicago Surgical and Electrical Co.) (B) the feeding stage covered with hamster skin. (C) the cage containing flies. (D1), (D2), (D3) 3 separate parts of cover, all painted black.

stage thus prepared on an electric heater (Fig. 2A) at a steady temperature of 35° C., and put on the skin surface a cage (Fig. 2C) containing the flies hatched in the laboratory during the last 24 hours. Arrange a set of covers (Fig. 2, D1, D2, D3) to keep the cage dark and to prevent the rising of heat from the heater.

Many flies may be put into one cage for feeding, but the best result is obtained when the number is not too large. In 30 minutes, 95% of the flies may be fully engorged if the number of flies in the cage does not exceed 20, whereas the percentage drops to 50 or 60 when the number of flies is 60 or more. There are always a number of flies which will not feed until they are 24 hours old. The feeding is best done in the evening, but is sometimes successful in day time, if the covers are properly set.

Flies fed by this method almost never die within the first 3 or 4 days of life. During the months of July and August, with an average temperature in the laboratory of 27° C. and a humidity of approximately 60%, the average span of life of these flies, unless refed on the fourth or fifth day, was 5 or 6 days. In order to lengthen this period to give the parasites longer time to develop, the flies were kept in an ice box at a temperature of approximately 21°

C. With refeeding some of the flies at this temperature lived for 18 days, and an abundant growth of flagellates in the flies were obtained.

For breeding purposes flies are fed on the whole blood of a normal hamster, on the same stage.

From July 16th to August 31st, 1928, 2131 female sandflies (1918 *Phlebotomus major* and 213 *Phlebotomus sergenti*), practically all that were hatched in our laboratory, were fed so satisfactorily that we feel justified in suggesting the adoption of this technique for the artificial feeding of sandflies. The method described is an attempt to imitate the natural conditions under which the flies feed, but the artificial conditions are better than the direct feeding on animals. The immobility of the feeding stage is a decided advantage, as the biting insects are frequently disturbed by the movements of the live animal. The ease with which the insect gets blood, practically every time the stylets pierce the skin, is another distinct advantage of this feeding stage.

The principle of this method might be used in experiments on transmission of diseases in which biting insects play a rôle. The skin preparation of the feeding stage might serve either as the source of infection, or as a medium for transmission.[2]

[2] Hu, C. H., Huie, Dorothy, and Lee, C. U., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi.

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Slapping as a Factor in Transmission of Kala-Azar by Sandflies (Phlebotomus).

C. H. HU, DOROTHY HUIE, AND C. U. LEE. (Introduced by Hsien Wu.)

From the Departments of Pathology and Medicine, Peking Union Medical College.

The results in the experimental transmission of kala-azar thus far have been uniformly negative.[1, 2] Shortt, attempting transmission by means of sandflies, attributes his negative results to insufficient susceptibility of the experimental animals rather than to inability of the fly to expel the flagellates from its buccal cavity into the wound while feeding. We are inclined to believe that the negative results

[1] Shortt, H. E., Barraud, P. J., and Craighead, A. C., *Indian J. Med. Res.*, 1927, xiv, 589.

[2] Young, C. W., and Hertig, Marshall, PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 823.