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).

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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.

might have been due to the overlooking of one natural factor not previously considered, namely, the crushing of the fly by slapping the itching skin, where the fly is feeding. To ascertain the importance of this factor in the transmission of kala-azar, a method has been worked out, imitating as far as possible the natural conditions under which the flies feed and are crushed.

The sandflies, *P. major,* are fed with the blood-parasite mixture on the feeding stage within 24 hours after hatching,[3] and are then kept in the ice box at 21° C. After 6 to 10 days, they are allowed to refeed and, while feeding, are crushed by slapping. This is occasionally done as late as the 18th day, if the flies have had a second feeding.

1. *The feeding stage.* This stage is like the one for the blood-parasite mixture, except that the dish is only ½ inch in diameter. The flannel is cut a little smaller than the dish and is cut in 2 equal halves. It is then pressed and saturated with defibrinated rabbit blood, and covered with a piece of skin from a normal hamster. Strictest asepsis is necessary in these preparations, but the heavily contaminated external surface of the skin is not sterilized for reasons which are given below. The prepared feeding stage is placed on the electric heater set at 35° C., and the slapper is adjusted to the skin surface.

2. *Feeding and slapping.* After the slapper is set with the lower end of the piston lifted by the magnet to a level ½ inch above the skin surface, a fly is introduced through the side-tube which is then plugged with cotton. With a bright electric light the fly is closely watched. When its abdomen is being engorged the switch for the electric magnet is turned off, the piston instantly drops, and crushes the fly while its biting parts are still in the skin. The fly is then immediately fixed for serial sections. The skin is removed from the stage and each of the two pieces of flannel is put into a separate tube of NNN media for the cultivation of flagellates. The cultures are examined at the end of 3 weeks. (Table I.)

The process described is time-consuming, for the flies must be fed and slapped one by one. Most of them do not feed, probably due to the sudden change in surroundings, and to the presence of a bright light. Those which do feed are not as fully engorged as when fed in the dark, usually stopping to suck when they show blood in the thorax or in the anterior abdomen.

In a second series of experiments the flies were allowed to with-

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

draw their stylets after feeding without being slapped, after which the flannels bitten by them were cultured. (See Table II.)

TABLE I.

A record of the flies slapped in the act of feeding and the result of cultures made from the flannels.

Culture No.	Date of hatching and first feeding	Interval between 1st feeding and slapping	Flagellates in serial sections	Culture of flannel A.	Culture of flannel B.	Remarks
15	Aug. 18	9 days	+	—	—	
16	" 18	9 "	+	—	—	
17	" 19	8 "	+	—	Cont.	
18	" 19	8 "	+	—	—	
20	" 18	10 "	+	—	—	
22	" 19	10 "	+	—	—	
23	" 19	10 "	+	—	Cont.	
24	" 17	12 "	+	—	"	
26	" 22	8 "	+	—	"	
28	" 25	6 "	+	—	+	
29	" 14	18 "	+	—	Cont.	Refed with rabbit blood on August 22.
30	" 18	14 "	+	—	—	Refed with rabbit blood on August 27.
32	" 26	7 "	+	—	—	
33	" 26	7 "	—	—	—	
34	" 27	7 "	+	—	—	
36	" 28	7 "	+	—	—	
37	" 28	7 "	—	—	Cont.	
39	" 29	7 "	+	—	—	
39a	" 29	7 "	+	—	—	
40	" 30	7 "	+	—	—	
41	" 30	7 "	+	—	—	
42	" 30	7 "	+	—	Cont.	
43	" 30	7 "	+	—	—	
44	" 31	8 "	+	—	—	
46	Sept. 2	8 "	+	—	—	
47	" 6	7 "	—	—	—	
48	" 6	7 "	+	—	—	

Cont. = Contamination by bacteria.

TABLE II.

A record of the flies not slapped and the results of cultures of the flannels.

Culture No.	Date of hatching and first feeding	Interval between first feeding and slapping	Date of death of fly	Flagellates in serial sections	Culture of flannel A.	Culture of flannel B.
19	Aug. 14	8 days	Aug. 27	+	—	—
27	" 22	9 "	" 31	+	—	—
35a	" 27	7 "	Sept. 8	+	—	—
38	" 28	7 "	" 8	+	—	—
38a	" 28	7 "	" 8	—	—	—
38b	" 28	7 "	" 8	—	—	—
44	" 31	8 "	" 10	+	—	—
45	" 31	8 "	" 12	+	—	Cont.
49	Sept. 6	7 "	" 13	+	—	—

Cont. = Contaminated by bacteria.

FIG. 1. *The Slapper.*

The upper end of the copper piston is screwed to an iron cap (A), the lower end is expanded, a little less than 3/8 inch in diameter, and projects 3/16 inch below the bottom of the glass tube. The glass tube is 2.5 inches long and 3/8 inch in its inner diameter. 1/4 inch above the lower edge of the tube is a short side-tube for the introduction of the fly. Around the piston is a steel-wire spring which pushes down when the current in the magnet (B) is broken.

Table I shows one positive experiment out of 27. Here a positive, non-contaminated culture of flagellates morphologically typical of Leishmann-Donovan bodies was obtained from one of the 2 flannel pieces on the feeding stage. Of the remaining 26 flies 23 contained flagellates, but failed to infect the flannel. Seven flannel pieces showed contamination by bacteria. Table II shows 9 experiments without slapping, all negative. Two of the 9 flies proved microscopically negative for flagellates. One flannel piece was contaminated by bacteria. All contaminations were due to direct introduction of bacteria through the hamster skin by the stylets. This statement is based upon the fact that in every case only one of the 2 flannel pieces was contaminated.

From the one positive culture (Table I) subcultures were made. We believe this positive finding to be significant, as demonstrating a means through which transmission of kala-azar might take place. As to the minute mechanism in play in our experiment we believe that the slapping, when undertaken at the right moment, causes a back flow of blood through the stylet all the way from the midgut. During the feeding there certainly are moments of relaxation of the crinkly posterior portion of the pharynx, making such a back flow possible.

Slapping does occur in nature as all know. If slapping is a neces-

sary factor, explanation for the apparent high immunity to kala-azar among infants might be found in as simple a fact as their inability to slap when bitten.

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Antipneumococcus Protective Substances in Normal Pig Serum.

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It has been generally held that natural immunity to the Pneumococcus, in common with other pathogenic micro-organisms, depends on a fundamental cellular difference of the host rather than on circulating antibodies.[1, 2] Bull and McKee,[3] however, showed that normal chicken serum was capable of protecting mice and guinea pigs against many times the fatal dose of virulent pneumococci. Furthermore, in a recent publication,[4] evidence was presented to indicate that a high concentration of antipneumococcus opsonins in the blood of naturally resistant mammals forms a common means of defense against pneumococcus infection. These findings would suggest that humoral defensive elements may play a much more important rôle in the mechanism of natural immunity than has hitherto been believed. This report presents further evidence in support of this view.

In the present study, serum from pigs, a naturally pneumococcus-resistant species, has been employed. Table I represents the protocol of an experiment in which white mice weighing about 20 gm. were each given intraperitoneal injections of 1 cc. of pig serum and 4 hours later varying amounts of an actively growing culture of a virulent Type I pneumococcus. Sera from susceptible animals, the rabbit and the guinea pig, were used as controls. It is clearly shown that normal pig serum protected mice against 10,000 times the minimum fatal dose while rabbit or guinea pig serum conferred no protection on mice against pneumococcus infection. Mice receiving less than 0.01 cc. of culture were protected by the pig serum, whereas 0.000,000,1 cc. of the same culture alone killed mice regularly.

[1] Zinsser, H., "Infection and Resistance," New York, 3rd edition, 1923, 68.

[2] Eastwood, A., *Rep. Pub. Health and Med. Subj.*, Ministry of Health, No. 22, 1923, 15.

[3] Bull, C. G., and McKee, C. M., *Am. J. Hyg.*, 1921, i, 284.

[4] Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1927, xlvi, 239.