

These experiments seem to indicate clearly that renal tissue undergoing destruction within the body contains a substance which causes marked elevation of both systolic and diastolic pressures if allowed to escape into the general circulation. This pressor substance cannot be demonstrated in the spleen under the same conditions and thus far is apparently specific for the kidney.

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The development of flagellates in Chinese sandflies (*phlebotomus*) fed on hamsters infected with *Leishmania donovani*.

CHARLES W. YOUNG and MARSHALL HERTIG.

[From the Department of Medicine, Peking Union Medical College, Peking, China.*]

Sandflies (*Phlebotomus*) have assumed a particularly important position in the study of the leishmaniases since Knowles, Napier and Smith¹ reported the appearance of herpetomonad flagellates in a large proportion of *Phlebotomus argentipes* fed on kala azar patients in Calcutta.

The present paper is a report of certain phases of studies undertaken on the sandflies of North China as possible transmitting agents of kala azar. Three species of *Phlebotomus* are known to us, namely, *Phlebotomus major* var. *chinensis* Newstead, and two unidentified species which we have designated *Phlebotomus* "B" and "C". *Phlebotomus* "B" is apparently the unnamed species mentioned by Newstead.² These three species occur in markedly variable proportions in several regions near Peking and Hsü-chowfu, Kiangsu.

In these studies (1) sandflies captured in houses of kala azar patients and elsewhere have been examined for flagellates. (2)

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¹ Knowles, R., Napier, L. E., and Smith, R. O. A., *Ind. Med. Gaz.*, 1924, lix, 593.

² Newstead, R., *Bull. Ent. Res.*, 1916-17, vii, 191.

Sandflies reared in the laboratory have been fed on kala azar patients and on hamsters heavily infected with *Leishmania donovani*. In the attempt to transmit kala azar these sandflies have been refed upon tested negative hamsters, and a certain number have been inoculated into other hamsters. As many as possible of such sandflies were examined for the presence of flagellates. A total of over 250 hamsters used in these transmission studies were all negative by liver puncture from 81 to 137 days after the experiments, but final results will be reported from autopsy findings. It is desired to report at this time (1) a technique of rearing sandflies and of feeding them on hamsters, with particular reference to the feeding of these insects a second time, a feature hitherto little studied, and (2) the results of examination for herpetomonad flagellates.

Technique. The method of rearing sandflies, which will be reported in detail in a separate paper, is a modification of the methods of Waterston³ and Smith.⁴ The breeding vessels are porous earthen pots with a thin lining of plaster of Paris. Over the top of the pot is tied a cover of bolting cloth pierced by a glass tube for the entry of the sandflies. The insects in the pot may readily be seen through the bolting cloth against the white plaster. The pot is set in a dish to which is added sufficient water to keep the plaster lining moist. Males and engorged females are introduced through the glass tube. Oviposition takes place on the plaster walls. The bolting cloth cover is removed as soon as the adults have died, and an earthen dish is inverted over the pot. Crushed feces of negative hamsters are supplied a few days before the estimated hatching of the eggs. The feces become somewhat moldy, but although a few of the very young larvae become entangled in the *fungus mycelia* the presence of the mold in our experience is an advantage rather than otherwise. After pupae are observed the bolting cloth cover is replaced. To obtain individual specimens or to transfer the adults from one vessel to another, they are first released in a cubical cage, provided with cloth sleeve, of the type commonly used for feeding winged insects, except that the cage is made of bolting cloth (No. 50, *i. e.*, fifty meshes to the inch) which permits the sandflies to be readily seen, with no possibility of their escape through the meshes.

³ Waterston, J., *Ann. Trop. Med. and Parasit.*, 1922, xvi, 69.

⁴ Smith, R. O. A., *Ind. J. Med. Res.*, 1925, xii, 741.

They may be caught against the walls of the cage in a small glass tube. This tube, which fits over the glass entry tube of the pots, is provided with a glass plunger by means of which the sandflies may be induced to run quickly through the tube into the pot.

Feeding Sandflies. The method of feeding sandflies which we have found most successful is the use of a feeding cage essentially the same as that used by Wolbach, Todd and Palfrey⁵ for feeding lice. This is made from a metal ointment box from which the centers of cover and bottom have been cut out. Bolting cloth (No. 50) is fastened over the bottom and another piece which serves as the cover is held fast when the metal cover is pressed into place. The edge of the box is bound with adhesive tape. The sandflies are introduced through a glass tube in the bolting cloth cover similar to that of the breeding pot. The Wolbach feeding cage is bound with adhesive tape to the forearm of a patient, and may safely be left over night. In the case of hamsters, the latter are made fast against a coarse screen by fine leather thongs around the four legs, and the feeding cage is applied to the closely clipped belly. Our routine was to leave the Wolbach cages on the patients over night, but for only two hours at a time on the hamsters. Occasionally a hamster in its wire cage was kept over night in the large bolting cloth cage into which sandflies had been released. The use of the Wolbach cage, however, was much more successful as well as more convenient.

EXPERIMENTAL WORK.

Material. *Phlebotomus "C"* apparently has two or more broods per season. Specimens were taken from the middle of June to the end of September. An abundant second generation, the progeny of wild sandflies, was reared in the laboratory during the summer. *P. major* var. *chinensis*, on the other hand, seems to be single-brooded, being found chiefly during June and the first part of July. Only a small fraction of these sandflies in the breeding pots completed their cycle during the summer and as a result laboratory-bred material of this species has been scanty. The experiments here reported were performed at Hsüchowfu during the season of 1925.

⁵ Wolbach, S. B., Todd, J. L., and Palfrey, F. W., *Etiology and Pathology of Typhus*, Cambridge, 1922.

Wild Phlebotomus "C". We have examined a total of 378 females and 48 males captured in two villages near Hsüchowfu from June 16th to September 30th. The majority of these were from kala azar houses. About half the specimens were living when taken for dissection, and the remainder, though dead, were still suitable for examination. Smears were negative for flagellates in all cases.

Feeding Experiments with Laboratory-bred Phlebotomus "C". The sandflies were allowed to engorge on kala azar patients or on heavily positive hamsters and were then allowed to feed on tested negative hamsters. The results of the first feeding on the infected host may be summarized as fractions in which the numerator is the number of females which actually sucked blood, and the denominator the total number of females given the opportunity to feed: Females fed on kala azar patients, 168/218; on positive striped hamsters 115/193; on positive giant hamsters 200/302; total 483/713.

The engorged females along with males were transferred to autoclaved breeding pots. Those which died were removed as promptly as possible and examined microscopically. The survivors after periods varying from one to thirteen days were given the opportunity to reengorge on tested negative striped hamsters. Of 286 thus given the opportunity, 140 are known to have sucked blood. Most of these refeedings took place four to seven days after the first feed.

Microscopic Examination. Of the 483 females of *Phlebotomus "C"* originally fed on kala azar patients or infected hamsters, 373 were dissected and examined microscopically, either before or after opportunity to refeed. A number were alive when taken for dissection. The remainder were dissected as soon after death as possible, usually within 24 hours, and the tissues were for the most part quite fresh. Herpetomonad flagellates were found in seven of the 373, either on fresh examination or in stained smears. The other 366 were entirely negative for flagellates and most of these were negative for bacteria or other organisms. These dissections were made from one to fourteen days after the potentially infective feed, the majority being after from three to ten days. The seven positive for flagellates were distributed as follows: four on the third day, and one each on the fourth, fifth and eighth days.

The 373 females examined had fed originally as follows: On kala azar patients, 141; on positive striped hamsters, 75; and on positive giant hamsters, 157. Of the seven containing flagellates, six had fed on giant hamsters and one on a striped hamster, while those fed on patients yielded none with flagellates.

Wild Phlebotomus major var. chinensis. Smears were made from sandflies captured in and near Hsüchowfu as follows: 16 females, 44 males, 12 sex not recorded. Microscopic examination was negative for flagellates in all cases.

Feeding Experiments with Laboratory-bred P. major var. chinensis. A total of 51 females engorged on either patients or positive hamsters out of 149 given the opportunity, as follows: Kala azar patients, 15/58; positive striped hamsters, 0/3; positive giant hamsters, 36/88. Of these 51 females, 31 were given the opportunity to refeed on tested negative striped hamsters. Seven are known to have refeed.

Smears were made from 45 of these 51 females. Herpetomonad flagellates, in some cases in great numbers, were found in 29 out of 34, or 85.3 per cent of those which had fed on positive giant hamsters, in marked contrast with the very small proportion, 3.3 per cent, which developed flagellates in the case of *Phlebotomus "C"*. These organisms occurred chiefly in the oesophagus or anterior portion of the mid-intestine, and occasionally in the oesophageal diverticulum. Eleven of those which had fed on kala azar patients were examined and were all negative for flagellates. The flagellate-containing sandflies were dissected from four to eight days after the infective feed.

Phlebotomus "B". This species is rare in the Hsüchowfu region, intensive collecting yielding only a few specimens in nearby villages. While this sandfly is known to feed on man in nature, our attempts to feed over 70 females on man and hamsters were entirely unsuccessful. Smears of 12 females and 9 males were negative for flagellates.

The herpetomonads which develop in sandflies fed on kala azar hosts have not as yet been definitely identified as *Leishmania donovani* by either the Calcutta workers or ourselves, though all evidence at present available renders such identity probable.