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Kala Azar Transmission Experiments With Chinese Sandflies (*Phlebotomus*).

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We reported¹ the development of herpetomonad flagellates in Chinese sandflies fed on hamsters infected with kala azar. In the present paper are summarized the results of various kala azar transmission experiments performed in 1925 and 1926.

The three species of sandflies, for the determination of which we are indebted to Professor R. Newstead, are: *Phlebotomus major* var. *chinensis* Newstead; *P. sergenti* var. Newstead (*Phlebotomus* "C" of our previous report), identical with an unnamed variety described from Mesopotamia²; *P. perturbans* var. Patton³ (*Phlebotomus* "B"). This latter species we have never fed on man or other mammals in the laboratory, but it readily engorges on toads.

In the following experiments we tested the hypothesis that *Phlebotomus* sandflies becoming infected by sucking the blood of kala azar patients are then able to transmit the disease to other persons by their bites. The first was fed upon heavily infected hamsters. Later they were fed upon tested negative hamsters.

As *P. sergenti* became infected with *Leishmania* only rarely, following "natural" feeds, as contrasted with the very large proportion of infected *P. major*, a technique described elsewhere⁴ was devised for feeding sandflies artificially with rich suspensions of Leishman-Donovan bodies. The apparatus consists essentially of a cork vise for immobilizing the sandfly and an adjustable holder for the feeding pipette, the end of which is fitted over the insect's stylets. Freshly defibrinated rabbit blood was centrifuged and the serum removed. Spleen tissue from a hamster heavily infected with *Leishmania* was ground up in this serum. A volume of spleen-serum suspension equal to that of the serum, originally removed, was then added to the rabbit corpuscles. The sandflies engorged readily. Infections with *Leishmania* were produced in all three species. Those for *P. perturbans* are not reported here, since feeding experiments on mammals have not proved possible with this species.

All the sandflies were laboratory-bred. The items are classified according to the number of days after the first, or potentially in-

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fective, blood meal of the sandflies concerned. "Positive" and "negative" sandflies indicate the results of examinations for *Leishmania* either in fresh preparations, stained smears or in sections. Hamsters bitten by positive sandflies represent tested negative striped hamsters from which at least one sandfly, demonstrated at dissection to contain *Leishmania*, is known to have sucked blood. Hamsters "exposed" are those cases in which no blood could be demonstrated in the sandflies after the feeding cages were removed. As visible blood was the criterion of feeding the number of hamsters classified as bitten represents actually the minimum subjected to the bites of potentially infected sandflies. Our routine was to keep the hamsters for 6 months after the experiment to allow ample time for an infection to become demonstrable. The animals were then killed with ether, and smears of the spleen, liver, bone marrow and heart-blood were examined microscopically. Negative findings in hamsters "dead within 90 days" are considered inconclusive and these are therefore indicated in a separate column. A number of hamsters injected intraperitoneally with positive sandflies developed heavy visceral infections of *Leishmania* (41 out of 180, or 23 per cent) thus demonstrating the pathogenicity of the organisms present in the sandflies, *but the entire series of experiments involving 413 hamsters bitten by or exposed to the bites of infected or potentially infected sandflies of two species without exception has resulted negatively.** Certain marked differences between *major* and *sergenti* are clearly shown. *Major* refeeds with reluctance, while *sergenti* refeeds readily. *Major* is very susceptible to infection with *Leishmania* following natural feeds (195 positive out of 348, or 56 per cent). Artificial feeds yield a particularly high proportion of infections (131 out of 157, or 83 per cent). The infection in *major* tends to persist. On the other hand, *sergenti* following natural feeds becomes infected only rarely (16 out of 661, or 2.4 per cent). The massive doses in the artificial feedings yield a greatly increased proportion of infections (169 out of 349, or 48 per cent), but in both naturally and artificially fed *sergenti* there is a tendency for the infection to die out after the fifth or sixth day.

In addition to hamsters injected with positive sandflies, 44 hamsters were injected intraperitoneally with 27 lots, aggregating 96 *sergenti* females, potentially infected but negative at dissection. No infections developed in any of the hamsters.

Five hamsters were inoculated by scarification with two *major*

* Completely negative results from a comparable feeding series have been obtained with *P. argentipes* by Shortt, Barraud and Craighead. *Ind. J. Med. Res.*, 1927, xiv, 589.

females containing flagellates of *Leishmania*. The hamsters were negative at autopsy.

260 female *sergenti*, taken mostly in kala azar houses were inoculated intraperitoneally into 53 hamsters. Control suspension smears made for each sandfly were all negative; 183 additional females, of which no control smears were made, were inoculated into 24 hamsters. Nine of the 77 hamsters died within 90 days. No infection developed in either these or the 68 survivors. 118 females and 48 males examined by smears only were negative. A lot of 353 females and 41 males of *sergenti* from the same group of houses were all negative. Thus a total of 904 females and 89 males of *sergenti* from a kala azar region examined by smears or by inoculation into hamsters or by both methods, have been negative for *Leishmania*. About 200 other sandflies, including both *major* and *sergenti*, were all negative for flagellates. A large proportion of *perturbans* were heavily infected with a crithidiform flagellates. No infection developed in 32 hamsters inoculated intraperitoneally with 124 such wild *perturbans*.

Sergenti is the dominant sandfly in kala azar villages near Hsü-chowfu but is infected with difficulty and such infections tend to die out. *Major*, on the other hand, while easily infected may be very plentiful in places where there is little or no kala azar. Neither species has been infected following feeds on kala azar patients, nor have infections with *Leishmania* been found in wild sandflies.

Summary. Although sandflies infected with *Leishmania* may produce infections in hamsters when inoculated intraperitoneally, all efforts to transmit the infection to hamsters by their bites have failed.

This is a preliminary communication.

¹ Young, C. W., and Hertig, M., *Proc. Soc. Exp. Biol. and Med.*, 1926, **xxiii**, 611.

² Newstead, R., *Bull. Ent. Res.*, 1913-14, **iv**, 309.

³ Patton, W. S., and Hindle, E., *Proc. Roy. Soc., Ser. B*, 1926, **c**, 405.

⁴ Hertig, A. T., and Hertig, M., *Science*, 1927, **lxv**, 328.