

the maximal difference between the native bactericidal action of dyes and that enhanced by light was at most 10 fold. It therefore appears that the parallelism of bacteria in their reactions to the gram stain and the susceptibility to photodynamic action, as suggested previously,¹ is again confirmed.

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Attempts to Infect *Ornithodoros moubata* with the Chinese Strain of *Spirochaeta recurrentis*.

LAN-CHOU FENG AND HUEI-LAN CHUNG. (Introduced by R. J. C. Hoeppli.)

From the Division of Parasitology, Department of Pathology, and Department of Medicine, Peiping Union Medical College, Peiping, China.

Biologically there are 2 groups of relapsing fever spirochaetes, one being transmitted by lice and the other by ticks, chiefly of the *Ornithodoros* group. The Chinese strain of *Spirochaeta recurrentis* has been demonstrated by Robertson¹ and Chung² to be transmitted by the louse, *Pediculus humanus corporis*, and its development in this insect has been studied recently by Chung and Feng.³ In order to see whether infection with the Chinese strain of relapsing fever spirochaetes can be established in *Ornithodoros moubata*, and whether infection can be transmitted to laboratory animals by these ticks, a series of experiments was carried out.

Four lots of young larval ticks and one lot of adult male and female ticks were fed on squirrels heavily infected with Chinese relapsing fever (30-40 spirochaetes to each oil immersion dark field of the fresh smear). After the infective feeding, the ticks were kept at a room temperature of 25-28°C. Examinations for infection of the ticks were made by dissection and searching for spirochaetes under darkfield illumination by the method used by Feng and Chung,⁴ by feeding on clean squirrels and finally by injecting emulsified ticks into squirrels. The blood of the animals was examined daily with dark ground illumination for spirochaetes throughout the course of the experiment.

The dissections, as shown in Table I, demonstrate that the spiro-

¹ Robertson, R. C., *Chinese Med. J.*, 1932, **46**, 853.

² Chung, H. L., *Chinese Med. J.*, 1936, **50**, 1723.

³ Chung, H. L., and Feng, L. C., *Chinese Med. J.*, 1936, **50**, 1181.

⁴ Feng, L. C., and Chung, H. L., *Chinese Med. J.*, 1936, **50**, 1185.

TABLE I.
Results of Dissection of Ticks Fed on Squirrel Infected with Chinese Strain of *Spirocheta recurrentis*. (These ticks were kept at 25-28° C.)

Days of Infection	No. Dissected	Stomach		Legs		No. of spirochaetes	
		No. +	No. —	No. +	No. —	Living	Dead
1	6	6	0	3	3	++++	+
2	8	8	0	1	7	++	+++
3	8	8	0	1	7	+	++
4	4	3	1	1	3	+	++
5	5	2	3	0	5	0	+
6	4	0	4	0	4	0	0
7	5	0	5	0	5	0	0
8	2	0	2	0	2	0	0
9	2	0	2	0	2	0	0
10	2	0	2	0	2	0	0

chaetes penetrated the stomach wall and reached the body cavity in some of the ticks during the first 4 days of infection. All the spirochaetes, including those which had already reached the body cavity and those still in the stomach gradually died, degenerated, and finally disappeared, beginning as early as the 4th day in some of the ticks. In a few instances, a small number of spirochaetes were found to survive until the fourth day of infection. From the 6th day onward spirochaetes could not be found at all in any of the ticks examined. In a few of the ticks dissected from the 1st to the 11th day of infection additional examinations for spirochaetes in other organs lying in the body cavity, including the salivary glands, the nerve ganglion, the coxal glands, the reservoir, and the malphighian tubes gave entirely negative results.

In the feeding experiments, since the ticks refused to suck blood again earlier than 4 days after the infective feed, and even after this date only a small number of them would suck blood at the same time, these experiments were extended over a period of 25 to 69 days in the hope that repeated bites during this period might produce an infection which would otherwise be missed.

In Table II it is shown that all 4 squirrels on each of which separate lots of 5-17 infected ticks were fed from 3-6 times with a total of 45-66 bites failed to contract relapsing fever and their blood remained constantly negative for spirochaetes as proved by daily examination extending 31-55 days after the last feed.

At the end of the feeding experiment 5 ticks from each of the 4 lots were ground up in 2 cc. normal saline solution and the emulsion injected subcutaneously into the negative squirrels used for the feeding experiment. Besides the subcutaneous injection, 4 drops of the emulsion were also dropped into the eyes and nose of each animal. On examination, 8 and 25 days respectively after the injection, 2

TABLE II.
Result of Feeding and Inoculation Experiments.

Squirrel No.*	Feeding of ticks on squirrels				Total No. of bites	Blood of squirrel	No. of ticks injected	Injection of emulsified ticks into squirrels	
	Days after ticks fed been infected	No. of feedings	No. of ticks fed each time	No. of ticks fed each time				Animals remained negative after injection	Animals remained negative after injection
1	66-116	5	9-12	52	No spirochaetes	5	25 days. Died		
2	7-32	4	8-14	45	" "	5	35 "		
3	19-58	3	14-17	45	" "	5	34 "		
4	4-73	6	5-15	66	" "	5	8 "		Died

* All the squirrels were splenectomized.

† On the last day 4 drops of infective blood were put into the eyes and nose of each of these squirrels, in whose blood spirochaetes were found 4 and 5 days afterwards.

animals which died showed no spirochaetes in the blood. Into the eyes and nose of the other 2 squirrels, whose blood was consistently negative for 33 and 34 days after receiving the tick emulsion, infected squirrel blood was dropped. They were found to take the infection, as spirochaetes were seen in their blood 4 or 5 days afterwards. This shows clearly that the squirrels used in the experiment were susceptible to the infection (Table II).

Attempts to transmit relapsing fever spirochaetes which are normally carried by lice by means of ticks to laboratory animals have been made by various workers. Manteufel⁵ stated that he was able to transmit the Russian strain of relapsing fever spirochaetes to rats through the bite of experimentally infected *Ornithodoros moubata*. Neumann⁶ also succeeded in transmitting both the American and Russian strains of *Spirochaeta recurrentis* to mice and rats through the bite of the same tick.

In the experiments recorded in the present paper infection with the Chinese strain of *Spirochaeta recurrentis* of Peiping could not be maintained in either young or adult *Ornithodoros moubata*. After the infective feed, some of the spirochaetes transversed the gut and reached the abdominal cavity of the tick. They failed, however, to enter the salivary glands, the nerve ganglion, the reservoir and the coxal glands.

At the temperature of 25-28°C. the spirochaetes in the stomach as well as those that had already reached the body cavity of the tick gradually died, degenerated and finally disappeared about 6 days after the infective feed. Feeding of such ticks on and injection of their emulsion into squirrels failed to produce relapsing fever in these animals which were subsequently proved to be susceptible.

These findings suggest that the Chinese strain of *Spirochaeta recurrentis* is biologically different from those transmitted by ticks. It also seems to indicate that this strain of the relapsing fever spirochaetes does not behave in the tick in the same way as the Russian and American strains which, according to the work of Manteufel and Neumann, can be easily transmitted to laboratory animals by *O. moubata*.

⁵ Manteufel, Arb. a. d. Kaiserl., *Gesundheitsamte*, 1908, **29**, 337.

⁶ Neumann, R. O., *Munch. Med. Wochr.*, 1909, **56**, 477.