in harmony with the observations of Sikora[5] but does not agree with the findings of Widmann[6] and Kisskalt,[7] who stated that chilling overnight at −5°C. or at −10°C. did not prevent eggs of lice from hatching.

Conclusion. Chilling for a sufficient period is an effective means of destroying both body-lice and their eggs. Where refrigerators having a temperature lower than −12°C. are available, the delousing of valuable furs and other delicate garments which would be damaged by moist or dry heat, can be safely and effectively achieved by this means.

9218

Photodynamic Action of Various Dyes on Bacteria.

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In the presence of ordinary visible light from an electric bulb, methylene blue was found to exert a rapid bactericidal action on certain bacteria which survived the same dye even in higher concentration in the absence of lamplight.[1] At the same time, gram negative bacilli were found to be highly resistant to this action of methylene blue. In continuation of a systematic study of photodynamic action of dyes on bacteria, various other common dyes have been chosen, and tests with representative gram positive and gram negative organisms repeated. The result of such a study is hereby presented.

Saline solutions of eosin, mercurochrome, acid fuchsin, basic fuchsin, and fluorescein, and a commercial 2% solution of trypaflavine were used. Bacteria were grown on either blood- or plain meat-infusion agar for 24 hours and were then suspended in saline. Except in the case of trypaflavine, which was diluted with the suspension to the desired concentrations, suspensions were added to equal parts of dyes in the different dilutions recorded in Table I.

In order to facilitate the study of a large number of specimens at the same time, the procedure previously employed was slightly

5 Da Rocha-Lima, H., und Sikora, H. *Handbuch der Biologischen Arbeitsmethoden*, 1925, **12**, 769.

6 Widmann, E., *Z. f. Hyg. u. Infek.*, 1915, **80**, 289.

7 Kisskalt, K., *Deut. med. Woch.*, 1915, **41**, 154.

1 T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 328.

modified. Instead of petri dishes, sterile, hollow-ground slides with 2 cells each were used. The cells were protected with coverslips which permitted maximal penetration of light from a 100-watt bulb 10 cm. distant. As in previous experiments, the slides were placed on a cooling machine which kept their temperature at or below 20°C. Controls were exposed to diffuse daylight at room-temperature. At intervals of 15, 30, and 60 minutes after exposure, samples were plated and examined for growth after 24 hours' incubation. The pertinent results of the photodynamic action of eosin, mercurochrome and trypaflavine after 60 minutes' exposure to lamplight are presented in Table I, while those from acid fuchsin, basic fuchsin, and fluorescein were omitted as these dyes were practically inert. The low solubility of the last two could account for their inactivity.

Besides the data presented in Table I, it may be mentioned that while 60 minutes of exposure revealed the maximal photodynamic action of the dyes, in the majority of instances however, a 30-minute exposure was almost as effective. Even with 15 minutes' exposure, *Pneumococcus* type I was killed by eosin and by mercurochrome at 1:10,000 dilutions of the dyes, and *C. diphtheriæ* by eosin at 1:10,000. However, the "prozone," in which higher concentration failed to kill while more dilute solutions did, was more noticeable after only 15 minutes of exposure. For instance, while no growth appeared in a suspension of *Pneumococcus* type I in 1:10,000 of eosin, some growth occurred from that of 1:1,000 and maximal growth from those from 1:10 and 1:100. This phenomenon was repeatedly seen in different types of cultures. It seems to suggest that in studying the photodynamic action of dyes on bacteria there is also an optimal dilution at which the dyes would act as it has been previously demonstrated with bacteriophage[2] and with toxin.[3]

Two facts stand out quite clearly from the above observations. In the first place, eosin was found to have a particularly effective photodynamic action—the difference between its native bactericidal action and that after exposure to light was more than 10,000 fold. In the case of methylene blue this difference was only 100 fold. Mercurochrome was more bactericidal in the absence of light, but its action on gram positive organisms was definitely enhanced by light. Trypaflavine behaved in a way intermediate between these 2 dyes. In the second place, it was remarkable that none of the dyes tested would act effectively against a gram negative bacillus, as

[2] Perdrau, J. R., and Todd, C., *Proc. Roy. Soc. B*, 1933, **112**, 277.

[3] Lin, F. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 656.

TABLE I.
Photodynamic Action of Eosin, Mercurochrome and Trypaflavine on Bacteria.

Organisms	Dilution of dye	Eosin		Mercurochrome		Trypaflavine	
		Exp.	Unexp.	Exp.	Unexp.	Exp.	Unexp.
Pneumococcus type I	1:10	+	++++	—	++++		
	1:100	++	++++	—	++++		
	1:1000	—	++++	—	++++	—	++++
	1:10,000	—	++++	—	++++	—	++++
	1:100,000	—	++++	—	++++	++	++++
	1:1,000,000	++++	++++	++++	++++	++++	++++
Streptococcus hemolyticus	1:10	—	—	—	—		
	1:100	—	+	—	+		
	1:1000	—	++	—	++++	+	±
	1:10,000	—	++++	—	++++	+++	++++
	1:100,000	++++	++++	++++	++++	++++	++++
	1:1,000,000	++++	++++	++++	++++	++++	++++
Staphylococcus albus	1:10	++++	++++	—	—		
	1:100	++++	++++	—	—		
	1:1000	++++	++++	—	—	—	++
	1:10,000	++++	++++	—	++++	—	++++
	1:100,000	++++	++++	++++	++++	+	++++
	1:1,000,000	++++	++++	++++	++++	++++	++++
S. paratyphi	1:10	++++	++++	—	—		
	1:100	++++	++++	—	—		
	1:1000	++++	++++	—	+	—	+
	1:10,000	++++	++++	+++	++++	+++	+++
	1:100,000					++++	++++
	1:1,000,000					++++	++++

C. diphtheriæ	1:10	—	++++	—	—		
	1:100	—	++++	—	—		
	1:1000	—	++++	—	—	—	+
	1:10,000	—	++++	—	++++	—	+++
	1:100,000	—	++++	++	++++	±	+++
	1:1,000,000	++++	++++	++++	++++	+++	+++
S. paradysenteriæ Flexner	1:10	++++	++++	—	—		
	1:100	++++	++++	—	—		
	1:1000	++++	++++	—	—	—	—
	1:10,000	++++	++++	++++	++++	—	++++
	1:100,000	++++	++++			++	++++
	1:1,000,000					++++	++++
Neisseria intracellularis	1:10	—	—	—	—		
	1:100	—	++++	—	—		
	1:1000	—	++++	—	++	—	—
	1:10,000	—	++++	—	++++	—	—
	1:100,000	—	++++	++++	++++	—	+++
	1:1,000,000	++++	++++	++++	++++	+++	+++
Br. abortus	1:10	++++	++++	—	—		
	1:100	++++	++++	—	—		
	1:1000	++++	++++	—	—	—	—
	1:10,000	++++	++++	++++	++++	—	—
	1:100,000			++++	++++	—	++
	1:1,000,000			++++	++++	++++	++++

— = no growth.
\+ = growth of less than 5 colonies.
++ to ++++ = increasing number of colonies.

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.

9219

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

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Biologically there are 2 groups of relapsing fever spirochaetes, one being transmitted by lice and the other by ticks, chiefly of the *Ornithodorus* group. The Chinese strain of *Spirochaeta recurrentis* has been demonstrated by Robertson[1] and Chung[2] 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 *Ornithodorus 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.