

the different sets of figures comparable, this figure of 17 hours should be added to that of the swabbed untreated animals. The corrected peak reaction would thus be at 63.4 hours. Delayed appearance of cornified cells in the vaginal smears of animals injected with estrogenic substance may therefore indicate a false positive reaction, since swabbing alone causes cornification.

The mechanism of cornification of the vaginal epithelium as induced by swabbing is not clear. Although swabbing apparently stimulates the process of cornification it does not prolong it indefinitely. The cornified cells usually disappear from the vaginal smear within 3 or 4 days after the first swabbing. In general, when epithelial proliferation and cornification follow local irritation, the process is progressive within limits and tends to be chronic.

*Summary.* Swabbing alone produces a significant cornification of the vaginal epithelium in a high percentage of ovariectomized rats. The changes thus induced come later than those following injections of estrogenic substance, and are transient, even though swabbing is continued. The reaction to swabbing materially affects the results of biological assay.

### 8361 P

#### Photodynamic Action of Methylene Blue on Bacteria.

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The effect of the prolonged use of photodynamically active dyes on a number of bacteria was summarized by Reitz.<sup>1</sup> Workers mixed the dyes and the cultures and let them stand for days exposed or unexposed to light. It was found that some dyes, including methylene blue, were more active in the presence of light than other dyes, such as fluorescein. It was also found that *B. diphtheriae* was more susceptible to such action than *B. typhosus*. Since then, however, very few reports have appeared. In view of the recent interest in the study of photodynamic action on viruses, bacteriophage,<sup>2</sup> and toxins,<sup>3</sup> it seems worthwhile to restudy this problem systematically.

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<sup>1</sup> Reitz, Adolf, *Zentralb. f. Bakt.*, 1908, **45**, 270, 374, 451.

<sup>2</sup> Perdrau, J. R., and Todd, C., *Proc. Roy. Soc. London*, 1933, **112**, 277, 288.

<sup>3</sup> Shortt, H. E., and Mallick, S. M. K., *Ind. J. Med. Research*, 1935, **22**, 529.  
Lippert, K. M., *J. Immunol.*, 1935, **28**, 193.

TABLE I.

Species of Bacteria	Final Concentration of Methylene Blue	Growth in	
		Exposed Mixture	Unexposed Mixture
<i>B. typhosus</i>	Saturated	++++	++++
	1-1	++++	++++
	1-10	++++	++++
	1-100	++++	++++
	1-1000	++++	++++
	1-10000	++++	++++
<i>B. coli</i>	Saturated	++++	++++
	1-1	++++	++++
	1-10	++++	++++
<i>B. dysenteriae</i> Shiga	Saturated	++++	++++
	1-1	++++	++++
	1-10	++++	++++
<i>B. abortus</i>	Saturated	+	+
	1-1	+++	+++
	1-10	+++	++++
	1-100	++++	++++
<i>M. catarrhalis</i>	Saturated	0	+++
	1-1	0	++++
	1-10	++	++++
	1-100	+	++++
	1-1000	++++	++++
<i>B. alkaligenes</i>	Saturated	0	++++
	1-1	0	++++
	1-10	++	—
	1-100	+++	—
	1-1000	++++	—
<i>V. cholerae</i>	Saturated	0	0
	1-1	0	0
	1-10	0	0
	1-100	0	0
	1-1000	0	+++
	1-10000	++	—
<i>Staphylococcus albus</i>	Saturated	+++	+++
	1-1	++++	++++
	1-10	++++	++++
	1-100	++++	++++
<i>Streptococcus hemolyticus</i>	Saturated	—	+++
	1-1	—	++++
	1-10	0	++++
	1-100	++	++++
	1-1000	++++	—
Pneumococcus type I	Saturated	—	+++
	1-1	—	+++
	1-10	0	++++
	1-100	+	++++
	1-1000	+++	—
	1-10000	++++	—
<i>B. diphtheriae</i>	Saturated	—	++
	1-1	—	+++
	1-10	0	+++
	1-100	0	+++
	1-1000	+++	++++

We have studied the effect of short exposures to light of different dilutions of methylene blue on a number of Gram-positive and Gram-negative microorganisms. A preliminary report is presented at this time.

Saturated aqueous solution of methylene blue was diluted to different concentrations with normal saline. Twenty-four hour broth cultures of different organisms were mixed with these solutions in Petri dishes. The bacteria-methylene-blue mixtures were exposed to an ordinary electric light of 100 candle power at a distance of 10 cm. for a period of half an hour. These Petri dishes were placed on a cooling machine which prevents the plate from getting too warm. Soon after the exposure, the organisms were plated out and incubated. The other portions of the same mixtures were not exposed and were similarly plated to serve as controls.

The experimental results are presented in Table I. The photodynamic action of methylene blue on bacteria varies a great deal. In general, the Gram-negative organisms seem to be more resistant than the Gram-positive ones. This parallelism of the reaction with the Gram stain is in conformity with that of bactericidal action of gentian violet in the absence of light<sup>4</sup> and the photodynamic action of a number of dyestuffs on bacteria in the presence of ultra-violet rays.<sup>5</sup>

It has also been found that the antigenicity of Pneumococcus Type I so treated was not lost, and that even after repeated exposures, a culture of *B. diphtheriae* did not lose its virulence. These subjects are now under study.

### 8362 P

#### Effect of Frequency of Stimulation on Tension Response of Muscle.

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The response of nerve, as measured by its heat production or summated action potential changes, has been shown by Bugnard and

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<sup>4</sup> Churchman, J. W., *J. Exp. Med.*, 1929, **16**, 221.

<sup>5</sup> Passow, A., and Rimpan, W., *Müch. Med. Wochr.*, 1924, **91**, 733.