

solution was reactivated by the addition of a small amount of the dialyzable fraction, the activity of the combined fractions therefore corresponded to at least 60 units of complement per cc, and (2) that the amount of the dialyzable component necessary for the reactivation was considerably less than that present in the original serum. As little as 0.8 cc of the greatly diluted solution was sufficient to restore activity. Therefore, one may conclude that the dialysate is a component of complement.

It is well known that certain components of complement may be altered or inactivated by the treatment of yeast,<sup>1</sup> ammonium hydroxide,<sup>2</sup> or oxidation with iodine.<sup>3, 4</sup> It was of interest to ascertain whether the dialyzable fraction corresponds to one of these fractions. We have tried to reactivate the complementary activity of sera treated with each one of the above 3 reagents, by adding to them varying amounts of the dialyzate; the results have been consistently negative. Furthermore, if the sera inactivated yeast, ammonium hydroxide or iodine were dialyzed separately, the resultant dialysate of each of the treated sera will reactivate the inactive dialyzed untreated serum, that of the oxidized serum being the least active. These results, therefore, indicate that the dialyzable component of complement is different from the so-called "third" or "fourth" or the oxidizable components of complement.

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### Influence of Color Filters on Photodynamic Action of Fluorescent Dyes on Gonococcus.

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In the study of photodynamic action, Tappeiner<sup>1</sup> found that with eosin the green rays were more lethal to paramecium than the other

<sup>1</sup> Osborn, W. W. B., *Complement or Alexin*, Oxford University Press, 1937, p. 16.

<sup>2</sup> Gordon, J., Whitehead, H. K., and Wormall, A., *Biochem. J.*, 1926, **20**, 1028, 1036, 1044.

<sup>3</sup> Chow, B. F., and Wong, Sam C., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 120.

<sup>4</sup> Chow, B. F., and Zia, Samuel H., *ibid.*, 1938, **38**, 695.

<sup>1</sup> Tappeiner, H. V., *Munch. Med. Wochenschr.*, 1900, **47**, 5.

parts of the spectrum. Later, Eidinow<sup>2</sup> demonstrated, however, that with the same dye the orange and yellow rays were more effective than the others. Hertel<sup>3</sup> in a more careful study showed that rays with different wave-lengths had different degrees of killing effect on the eosin-sensitized *B. coli* and paramecium. Most of the studies were made only with protozoa and *B. coli*, so it was thought of interest to apply similar tests to a pathogenic bacterium. In this study we have chosen gonococcus for experiment and in addition to eosin a few of the other dyes were also employed.

Different dilutions of eosin, methylene blue, trypaflavine, mercurochrome, protargol, argyrol, dimethyl-paraphenylenediamine hydrochloride, and tetramethyl-paraphenylenediamine hydrochloride were made with doubly distilled water, in 10-fold dilutions from 1:10<sup>2</sup> to 1:10<sup>10</sup>. The method of exposure to the ordinary light is the same as previously described.<sup>4</sup> However, in the experiment with filtered light the cells of the hollow-ground slides were covered with, instead of the ordinary thin cover-slips, different color-filters made by the Eastman Kodak Co. The filters used were: red (F. No. 29), orange (G. No. 15), yellow (K-1 No. 6), green (B. No. 58), light blue (H. No. 45), blue (C. No. 49), and violet (D. No. 35). At intervals of 5, 10, 15, 30, 60 and 120 minutes after exposure, samples from both exposed and unexposed specimens were plated and examined for growth after 24 hours' incubation. The results of the photodynamic action of eosin, methylene blue, trypaflavine, and mercurochrome at dilutions from 1:10<sup>5</sup> to 1:10<sup>9</sup> after 60 minutes' exposure both to filtered and unfiltered lights are presented in Table I while those of the other dyes are omitted since it was found that their germicidal properties were not enhanced by the action of light.

In addition to the data presented in Table I, it may be stated that with unfiltered light the effect of photodynamic action of methylene blue and trypaflavine for gonococcus was first manifested at the end of 5 minutes' exposure in a dilution of 1:10<sup>5</sup> while that of eosin and mercurochrome was found to appear first at the same time in the dilutions of 1:10<sup>2</sup> and 1:10<sup>4</sup> respectively. At the end of 120 minutes' exposure the bacteria were almost entirely killed by all the dyes at the dilution of 1:10<sup>9</sup> while in the control tubes they were only slightly attacked at the dilution of 1:10<sup>8</sup>.

With filtered lights, the results were more complicated, but it can be stated that the photodynamic action of all the dyes used here after

<sup>2</sup> Eidinow, A., *Brit. J. Radiol.*, 1930, **3**, 113.

<sup>3</sup> Hertel, E., *Z. allge. physiol.*, 1905, **5**, 95.

<sup>4</sup> T'ung, T., and Zia, Samuel H., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 326.

TABLE I  
Photodynamic Action of Mercurochrome, Trypaflavine, Eosin, and Methylene Blue on Gonococcus at End of 60 Minutes' Exposure to  
Both Filtered and Unfiltered Lights.

Name of dyes	Dilution of dyes	Exposed, filtered lights					Exposed, unfiltered light	Unexposed control
		Red	Orange	Yellow	Green	Light blue	Blue	Violet
Methylene blue	1:105	—	—	—	—	—	—	++
	1:106	++	—	—	—	—	—	+++
	1:107	+++	—	—	—	—	—	+++
	1:108	++++	++	++	++	++	++	+++
	1:109	++++	++	++	++	++	++	+++
Eosin	1:105	—	—	—	—	—	—	++
	1:106	—	+	—	—	—	—	++
	1:107	++	++	++	—	—	—	++
	1:108	++	++	++	—	—	—	++
	1:109	++	++	++	—	—	—	++
Trypaflavine	1:105	—	—	—	—	—	—	++
	1:106	—	—	—	—	—	—	++
	1:107	—	—	—	—	—	—	++
	1:108	++	—	—	—	—	—	++
	1:109	++	++	++	++	++	++	++
Mercurochrome	1:105	—	—	—	—	—	—	++
	1:106	—	—	—	—	—	—	++
	1:107	—	—	—	—	—	—	++
	1:108	—	—	—	—	—	—	++
	1:109	—	—	—	—	—	—	++

60 minutes' exposure is almost as effective as that after 120 minutes.

From Table I it is clear that 3 facts stand out very obviously. First, the gonococcus is particularly sensitive to the photodynamic action of the various dyes. This is not only in sharp contrast to the gram-negative bacilli but also to the closely related species such as meningococcus and *Micrococcus catarrhalis* which are more resistant than the gonococcus.<sup>4, 5</sup> Secondly, the effectiveness of the absorbed light in setting up the photodynamic action of the different dyes is almost as good as that of the unfiltered light. The only exception to this is found in the trypaflavine in which the unfiltered light is particularly effective, so much so that it even can bring about a complete destruction of gonococcus at the dilution of 1:10<sup>10</sup> at the end of 60 minutes' exposure. Lastly, each of the dyes has its special absorption-band in the spectrum. Thus for methylene blue and trypaflavine, the orange rays are the most effective and in the eosin, the green and light blue rays are the effective parts. For mercurochrome the orange, yellow, and violet rays seem to act equally well.

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**Potassium Tellurite and Copper Sulphate in Sabouraud's Medium for Isolation of Pathogenic Fungi.**

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In the isolation of pathogenic fungi, one of the principal difficulties is the occurrence of bacterial contamination. This is particularly true when dealing with scales and crusts which are usually loaded with saprophytic bacteria. In trying to overcome this difficulty we have found potassium tellurite and copper sulphate to be of value as by incorporating them into Sabouraud's medium they are found to be capable of suppressing the growth of bacteria but not that of fungi. In reviewing the literature, we have been able to find few reports in which chemicals were incorporated in Sabouraud's medium for the inhibition of contaminants. Sauthof<sup>1</sup> found

<sup>5</sup> T'ung, T., Proc. Soc. EXP. BIOL. AND MED., 1935, **38**, 328.

<sup>1</sup> Sauthof, Z. G., Dermat. Wchnschr., 1935, **101**, 1245.