treatment of the organisms with ether-alcohol was necessary in order to render the cells suitable to the extractive action of weak alkali.

Conclusions. A method suitable for the isolation of a type-specific protein in *C. diphtheriæ* is described. Both the lipoid and carbohydrate fractions are group-specific while the alkali-soluble protein is type-specific. The type-specific substance is heat-labile, being converted into a group-specific protein by heating at 56°C for 30 minutes.

11064

Bactericidal Action of X-Rays in the Presence of Dyes.

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Many dyes undergo color-changes when exposed to X-rays or some forms of visible light.[1] This indicates a process of oxidation-reduction. From this and other considerations it is generally assumed that the bactericidal effect of photosensitization is due to the oxidation of microörganisms.[2] It is conceivable that a non-lethal dose of X-rays in the presence of a dye may exhibit a similar function as that shown by visible light. This view is borne out by the following investigation.

Pneumococcus Type I, *Streptococcus hemolyticus, Staphylococcus aureus, B. subtilis, Mycob. phlei* and *Shigella paradysenteriæ* Flexner, *E. typhi, E. coli* and *Pseudomonas aeruginosa* were chosen as the representative gram-positive and -negative microörganisms. Mercurochrome, eosin, methylene blue, crystal violet, and safranin O were employed. The final concentration of mercurochrome and eosin was expressed in term of percent of the dye while that of methylene blue, crystal violet and safranin O, on account of their poor solubility, was expressed in terms of their saturated aqueous solution. The technic previously reported[3] for making the mixture of dye and organisms was followed. In order to expose a number of dye-bacteria mixtures in a limited space accessible to the X-rays, they were put into small glass cups of 1 cc capacity so that 10 of them could be exposed at the same time.

1 Clark, G. L., and Fitch, K. R., *Radiology*, 1931, **17**, 285.

2 Blum, H. F., *Physiol. Rev.*, 1932, **12**, 23.

3 Tung, T., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 326.

The X-rays were produced with a Douglas therapy-tube at 100 KV and 6 MA. The anode of the tube was cooled by an oil-circulating system. With this cooling system the temperature of the irradiated substance never exceeded 38°C. No filter other than the wall of the X-ray tube was used. The distance between the target and the dye-bacteria mixture was 10 cm; 250 r could be delivered to the mixture in one minute. After exposure the exposed and control mixtures were plated and incubated for 48 hours.

X-rays alone in doses of 15,000 r exerted no lethal action on pneumococcus Type I, *Streptococcus hemolyticus, Staphylococcus aureus, B. subtilis, Mycob. phlei, Shigella paradysenteriæ* Flexner, *E. typhi* and *E. coli.* However, when dyes were mixed with the suspensions the same amount of X-rays produced a significant lethal action on some of the microörganisms. This indicates that under suitable experimental conditions X-ray is a powerful germicidal agent. It was found that for gram-positive organisms mercurochrome was the most effective dye in the presence of X-rays. Exposure of pneumococcus Type I and *Streptococcus hemolyticus* to 15,000 r in 60 minutes resulted in complete inactivation of the organisms. Comparative bactericidal tests performed under the same conditions between X-ray-dye-organism and dye-organism system showed that the former was 1000 times more active than the native bactericidal action of the latter alone. The bactericidal action of eosin and methylene blue for these two organisms when exposed to the same amount of X-rays was 100 and 10 times respectively more than their native bactericidal effect. These two dyes, however, produced only slight lethal action on *Staphylococcus aureus* even when the amount of exposure was doubled. Similarly, eosin and crystal violet produced no bactericidal action on *B. subtilis* and *Mycob. phlei* respectively.

Since X-rays in the presence of dyes have exerted a significant bactericidal effect on some of the gram-positive organisms, the question naturally arises as to the possibility on inactivation of gram-negative organisms by the same process. It was found that in using the same dose of X-rays safranin O and eosin exerted a bactericidal effect in 1-100 and 1-10 dilution respectively on *Shigella paradysenteriæ* Flexner, *E. typhi* and *E. coli.* Both safranin O and eosin could kill *Pseudomonas aeruginosa* in 1-10 dilution when 7,500 r were used in half an hour. It may be of interest to mention that doses of X-ray exceeding this value were lethal to this organism in the absence of any dye.

While the photodynamic action on microörganisms could be en-

hanced by the addition of an oxidizing agent[4] the increase of the bactericidal power of X-rays in the presence of dyes could also be demonstrated by a similar treatment. It was found that eosin, after being exposed to 15,000 r in the dilution of 1-100, could not kill *Staphylococcus aureus,* but was able to kill this organism in a dilution of 1-100,000 when 1% hydrogen peroxide was introduced into the dye-bacteria mixture. Control experiments showed that eosin together with hydrogen peroxide in the absence of X-rays or hydrogen peroxide in the presence of X-rays did not produce any bactericidal effect.

In comparing the results of the present study with those of the previous investigation[3] on the photodynamic action of various dyes on microörganisms, certain similarities and differences can be observed. In the first place, pneumococcus Type I is the most susceptible organism to both photoinactivation and the combined action of X-rays and dyes while *Staphylococcus aureus,* spore-bearing and acid-fast organisms were the most resistant. In the second place, the bactericidal power of eosin in conjunction with either visible light or X-rays can be greatly increased by the addition of an oxidizing agent. On the other hand, eosin was found to possess little or no photodynamic activity on gram-negative organisms and yet in the presence of X-rays it exerted distinct bactericidal effect on organisms of the dysentery-typhoid-colon group. Similarly, eosin and safranin O which were proved to be potent photodynamical substances on gram-positive and -negative organisms respectively when exposed to visible light, were found to exert a weaker bactericidal action in the presence of X-rays.

The fact that a non-lethal dose of X-rays exerted bactericidal action in the presence of dyes suggests that the process involved is analogous to that of photodynamic action. It is to be noted, however, that, with the present amount of X-rays, the germicidal effect of the former is inferior to that of the latter.

Summary. Mercurochrome, eosin, and methylene blue exerted a bactericidal action on gram-positive organisms concurrently exposed to X-rays. While eosin is not a photodynamically active substance on gram-negative bacteria it produced a distinct bactericidal action on them in the presence of X-rays. The facts that a non-lethal dose of X-rays produced germicidal action in the presence of dyes and that such an action could be enhanced by introducing an oxidizing agent suggests that the fundamental mechanism of photosensitization and the combined action of X-rays and dyes may fall into the same category.

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[4] Tung, T., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 415.