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BY

FRANKLIN I. HARRIS AND HUBBARD S. HOYT

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FRANKLIN I. HARRIS AND HUBBARD S. HOYT

(From the Department of Pathology and Bacteriology, University of California)

Within recent years considerable interest has been shown in the study of the ultraviolet radiations in relation to their toxicity for living protoplasm. It was early found that these radiations exert a highly toxic influence on protoplasm exposed to them. Henri,[1, 2] in conjunction with various co-workers, has done pioneer work in this field, and was the first worker to point out the possibility of a practical application of this subject, namely, in the sterilization of various substances and solutions. Further work on the germicidal effect of ultraviolet light has been done by Houghton and Davis,[3] who found that the rays produced by the Cooper-Hewitt mercury arc exert a strong bacterial action on various species of bacteria, including spore forming organisms.

The action of these radiations was recognized as a photochemical process, and was supposed to be due to the absorption of the rays by the bacterial protoplasm. In a previous report[4, 5] we studied this phase of the problem and proceeded on the basis of the first law of photochemical action, that in a photochemical system, to be effective, the rays must be absorbed, usually by specific constituents. We have further shown that the toxic

action of ultraviolet light obeys this law and is due to the absorption of the rays by certain constituents of all living protoplasm, namely, the tyrosine and phenylalanin radicals of the protein molecules. These two acids are the specific absorbents in living protoplasm for the rays of the mercury arc.

The object of the present work was to confirm this work, using bacteria as a biological test, and further to study the relative speed of absorption of the ultraviolet rays by the protoplasm of the various types of bacteria.

Methods

Three organisms were selected as typifying three general groups of bacteria, which are classed on the basis of the possession or lack of protective structures, spores and capsules.

1. A non-sporulating, non-capsulated organism, *Staphylococcus aureus*.
2. A sporulating, non-capsulated organism, *Bacillus subtilis*.
3. A capsulated, non-sporulating organism, *B. mucosus capsulatus*.

For the exposure of these organisms various methods were tried. The organisms were grown on agar slants for twenty-four hours, and then washed off with sterile 0.85% NaCl. A preliminary exposure was made with a given amount of each saline suspension exposed directly to the rays. As no consistent results were obtained by this method, due undoubtedly to the absorption of the rays by the upper layers of the bacterial suspension, this method was abandoned.

A number of plating methods were then tried. Melted agar was poured at 42° C and allowed to harden in ten centimeter petri dishes. To prevent condensation it was found best to cover with sterile tile covers. After hardening three methods of inoculation were tried.

a. Two separate streaks were made with a loop full of saline suspension, one on either side of the diameter of the plate. Half of the plate was then exposed, the other half being protected by a glass cover, covered with black paper. The plate was then

incubated twenty-four hours, and the colonies were identified directly, or, in suspicious cases, smears were made. The objection to this method was that there was no certainty that the control streak and the exposed streak were similarly inoculated.

b. To overcome this objection a single large streak in the shape of the letter "Z" was made of one loopful of bacterial suspension, and half the plate exposed as before.

c. Another plating method experimented with was to flood the entire plate with a definite amount of saline suspension and then expose one side, as in the above.

Plating methods were also abandoned finally because they did not yield uniform results, due undoubtedly to some organisms getting in under the agar and being protected by the protein material.

The method finally employed was a cover slip method suggested by Professor Ivan C. Hall. Upon one surface of a sterile cover slip one loopful of saline suspension of a twenty-four hour agar growth was placed and allowed to dry in a sterile petri dish. Assuming the saline suspension to be uniform each cover slip therefore had approximately the same number of organisms. When dry the cover slips were exposed directly to the rays by placing them in a petri dish 12 cm. below the arc of the Cooper-Hewitt machine. After the given exposure the cover slip was picked up with sterile forceps, dropped into a tube of broth, the broth was incubated for forty-eight hours, and the results observed. The growth of these three organisms in broth is quite characteristic, and no further examination was usually necessary. In doubtful cases agar plates were streaked from the broth and the organisms were identified by the usual methods.

The exposures varied from five seconds to 200 seconds. Somewhat over 100 exposures were made, and although there were slight discrepancies in the results, due to the objections mentioned to plating methods, consistent results were obtained by the cover slip method, so that we may definitely say that:

Bacillus mucosus capsulatus was killed after 20 seconds.
Staphylococcus " " " 90 "
B. subtilis " " " 150 "

These figures represent the relative resistance of these three organisms.

The protective action of the amino acids was then studied. The cover slips were exposed as before, but between the cover slip and the arc a quartz beaker containing the given amino acid was interposed, so that the rays before striking the organisms passed through the amino acids. The results obtained confirm our previous work. With *B. subtilis*, whose normal extermination period is 150 seconds, we found exposure for forty minutes to ultraviolet light passed through 1% tyrosin solution exerted no toxic effect upon the bacilli, a good growth being obtained in forty-eight hours. Similarly, *Staphylococcus aureus* gave good growth after forty minutes; *B. mucosus capsulatus*, though not tested after longer exposure, gave satisfactory growth after ten minutes.

A good growth was also obtained with amino-benzoic acid after exposure of these organisms for 3200 seconds to ultraviolet light detoxicated by passing through this substance. Phenylalanin could not be secured, but there is little doubt that similar results could be obtained with it.

These results confirm those of our previous report[4,5] and indicate that the aromatic amino-acid radicals are the absorbing substances in bacteria as well as in protozoa. Kober's[6] work placed the absorption band for tyrosin at 248 to 297μ, or 2480–2970 Angstrom units, of wave length, and for phenylalanin 236–271μ, or 2360–2710 Angstrom units.

Therefore ultraviolet light, of wave lengths 2360–2970 Angstrom units, should contain practically all of the rays toxic for protoplasm. Two recent papers have appeared, however, which report different results. Browning and Russ[7] found the toxic action of ultraviolet light falling off sharply at 2960 A.U., which would apparently correspond with one edge of the tyrosine band. They report, however, an apparently constant toxicity from 2960–2100 A.U., and did not investigate below 2100 A.U. Newcomer[8,9] reported also a constant toxicity from 2100 A.U. up to a little less than 2900 A.U., the toxicity falling off to practically zero at 2970 A.U. The region in which tyrosine and phenylalanin

are both absorbed, i.e. 2480 to 2710 A.U., should be the most toxic for protoplasm, whereas the region containing wave lengths shorter than 2300 A.U. should be relatively non-toxic.

Our former experiments conclusively demonstrated that a solution of tyrosine will absorb practically all of the toxic rays, those getting through not being sufficiently toxic to kill paramecia after exposure for forty minutes, whereas if the rays absorbed by tyrosine were allowed to act the paramecia were killed in 100 seconds.

Conclusions

1. The aromatic amino acid radicals are among the substances in bacteria affected by the action of ultraviolet light, as was shown for paramecium in a previous report.

2. The ultraviolet radiations produced by the mercury arc of wave lengths not absorbed by tyrosine and phenylalanin are relatively non-toxic. Therefore, using Kober's determinations for the wave lengths corresponding to these two absorption bands, the ultraviolet radiations which are toxic for protoplasm are of wave lengths from 2480–2710 A.U.

3. In the three types of bacteria studied capsulated organisms were found to be most susceptible and sporulating organisms most resistant to the action of ultraviolet light. The work suggests strongly that the protoplasm of *Bacillus mucosus capsulatus* contains greater amounts of the above mentioned substances than the non-capsulated staphylococcus and the sporulating hay bacillus.

Transmitted February 6, 1919.

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