ULTRA-VIOLET LIGHT AND VACCINE VIRUS.

II. The Effect of Monochromatic Ultra-Violet Light upon Vaccine Virus.

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It has been known for many years that ultra-violet light is bactericidal. Finsen and Dreyer (1), in 1903, were probably the first to show that light with short wave-lengths is virucidal also. More recently (2–7) other reports have appeared dealing with the effects of ultra-violet irradiation upon bacteria, viruses, and enzymes. For the most part, however, single wave-lengths have not been used in the investigations, nor have the workers determined or taken into consideration the amount of energy required to kill or to inactivate these agents at single wave-lengths in the active ultra-violet region. One of the present authors (Gates) is making a quantitative study of the action of ultra-violet light on certain active agents in the hope of obtaining additional information concerning their nature. In the present paper the results of the studies concerning the effects of ultra-violet irradiation upon one of these active agents, vaccine virus, will be presented.

Methods and Materials.

Vaccine Virus.—The source and preparation of the vaccine virus have been described in the preceding paper. Fresh virus, however, instead of glycerolated material, was used in the experiments to be reported in the present paper.

Method of Irradiating and Testing the Virus.—Three drops of fresh virus were allowed to spread over the surface of 2 per cent nutrient agar (pH 7.4) in a small Petri dish. After being surface-dried in a horizontal position, certain areas of these plates were exposed for various intervals of time before the exit slit of a large quartz monochromatic illuminator to the different spectral lines of a quartz mercury arc in the ultra-violet region between λ 2302 and λ 3126 Ångström units. The intensity at each wave-length (measured in ergs per sq. mm. by means of a

standardized thermopile and high sensitivity galvanometer) multiplied by the time of exposure gave the total incident energy for each exposure. For comparison, a thin suspension of *Staphylococcus aureus* was washed over the surface of nutrient agar also. After surface drying, certain areas of the plates were exposed to the same spectral lines, and to intensities similar to those used for the vaccine virus.

After exposure to the light, small sections of the agar covered with vaccine virus were removed from exposed areas and from unexposed (control) areas, were emulsified with Locke's solution, and both were injected in aliquot doses into the shaved skin of the same susceptible rabbit. The agar plates with the staphylococci were incubated, and the bactericidal effect of the ultra-violet irradiations upon the bacteria was determined by counting the number of colonies in each exposed area and comparing them with the number in unexposed areas of the same size. In this manner figures were obtained which made it possible to express the effect of the light upon the bacteria in percentage of bacteria killed. In dealing with vaccine virus, however, the methods of titration are very crude. Consequently the determinations of the amount of virus inactivated or killed by the light are only relatively accurate, for the readings only indicated a positive or negative skin reaction. Thus the end-point had to be reached each time before a reading could be relied upon, *i.e.*, the virus had to be exposed long enough so that no visible reaction occurred at the site of injection in the skin. Under these conditions, it was impossible to say whether a subinfecting amount of virus remained active after exposure to the light or whether more energy had been used than was necessary to inactivate all of the virus.

A number of experiments were performed in the manner described (1) to determine and (2) to compare the effects that monochromatic ultra-violet light has upon vaccine virus and upon staphylococci. The results are summarized in Table I and graphically presented in Chart 1. In similar bactericidal studies on *S. aureus* carried out within a few months of these experiments closer approximations to the actual amount of incident energy involved in the destruction of all the exposed organisms were obtained. For comparison these figures are included in the table under Staphylococcus II and the curve derived from them has been superimposed upon the cross-hatched areas of the chart.

The upper line in Chart 1 represents the amount of energy in ergs per sq. mm. at each wave-length adequate, or more than adequate, to reduce the quantity of active vaccine virus below the dose necessary to produce a visible lesion in the skin of a susceptible rabbit. The lower line connects observations obtained from experiments in which

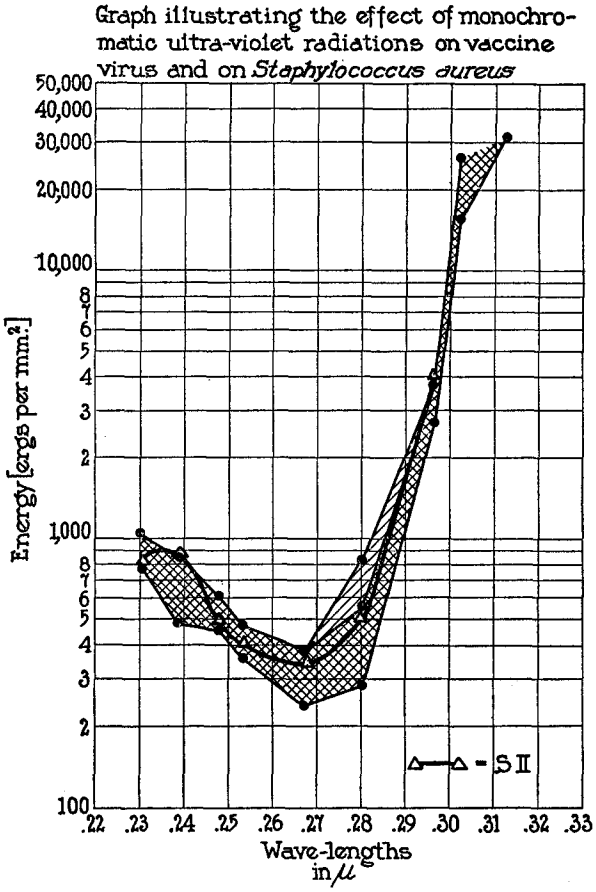

CHART 1. The incident energy required to inactivate all of a given specimen of vaccine virus lies between the upper and lower limits of the area cross-hatched ////. The energy necessary to kill all of the staphylococci falls within the area cross-hatched |||| . It is evident that these areas are identical except in the case of λ 2804. At this wave-length an amount of energy that killed all of the staphylococci failed to inactivate all of the vaccine virus. Only a "lower limit" is given at λ 3126. With the amount of energy used at this wave-length neither vaccine virus nor *Staphylococcus aureus* was seriously injured. It is not known what very large amount of energy, if any, at this wave-length is injurious to the active agents studied. The curve marked S II (*Staphylococcus aureus*) represents a closer approximation of bactericidal energy as found in the experiments of another series.

the amount of energy used was not sufficient to reduce the quantity of active virus to a point below the threshold dose. In regard to staphylococci, it was found (1) that at each wave-length, except one, the amount of energy inadequate to inactivate all the virus killed only from 62 to 76 per cent of the bacteria and (2) that the energy sufficient to inactivate the virus completely also killed all of the staphylococci.

The actual curves of the bactericidal (*Staphylococcus aureus*) and the virucidal (vaccine virus) effects of ultra-violet light lie within the shaded area, which doubtless could be reduced considerably in width by further experiments. Whether the curves for vaccine virus and for

TABLE I.

Amount of Energy at Certain Wave-Lengths Sufficient to Inactivate or Kill Vaccine Virus and Staphylococcus aureus.

Wave-length	Ergs per sq. mm.		
	Vaccine virus	Staphyloccus I	Staphyloccus II
2303–30	1,040	1,040	830
2379–97	856	856	900
2482	619	619	480
2536	480	480	410
2675	356	356	345
2804	858	572	510
2967	3,708	3,708	4000
3022	26,600	26,600	—
3126	30,600 (no effect)	30,600 (no effect)	—

staphylococci are identical cannot be determined with the crude methods available at present for titrating the virus. More significant than the absolute energies involved is the general shape of the curves which might be plotted within the shaded area. A rapid decrease in the required energy between λ 3022 and λ 2804 Ångström units, a nadir at λ 2675, and a rise towards λ 2302, corresponds closely to the curve representing the absorption of ultra-violet light by protein substances, and, in general, to the curves symbolizing various reactions of protoplasm or protein derivatives of protoplasm to light of short wave-lengths. The fact that these curves are similar is interesting and suggestive. Inasmuch, however, as the vaccine virus in a testicular

emulsion is admixed with a great quantity of animal protein to which it is probably adsorbed, one should be careful not to draw final conclusions from such observations as are reported in the present paper.

SUMMARY.

Under the experimental conditions described in the present paper, it was found that the amount of energy required to kill staphylococci at single wave-lengths in the active ultra-violet region was approximately the same as that necessary to inactivate vaccine virus.

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