

THE EFFECT OF MONOCHROMATIC ULTRAVIOLET RADIATION ON THE INFECTIVITY AND HEMAGGLUTINATING ABILITY OF THE INFLUENZA VIRUS TYPE A STRAIN PR-8¹

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The ultraviolet inactivation spectra of different viruses and bacteria, of a yeast, and of spores of a fungus show peaks in sensitivity at certain wave lengths. These peaks have been interpreted to represent the effect of monochromatic ultraviolet radiation on certain substances of biological importance in these microorganisms.

The following microorganisms show highest sensitivity at about 2,650 Å: vaccine virus (Rivers and Gates, 1928), *Staphylococcus* bacteriophage (Gates, 1934), influenza virus (Hollaender and Oliphant, 1944), *Escherichia coli* bacteriophage (Fluke and Pollard, 1949), *Staphylococcus aureus* (Rivers and Gates, 1928; Gates, 1929–1930; Ehrisman and Noethling, 1932), *E. coli* (Wyckoff, 1931–1932; Hollaender and Claus, 1935–1936; Hollaender and Duggar, 1936), *Pseudomonas pyocyaneus*, *Micrococcus candidans*, and *Vibrio* Finkler (Ehrisman and Noethling, 1932), *Saccharomyces cerevisiae* (Ehrisman and Noethling, 1932; Oster, 1934–1935), and spores of *Trichophyton mentagrophytes* (Hollaender and Emmons, 1939). (For *E. coli* Ehrisman and Noethling report the maximum at 2,510 Å.)

Rous sarcoma virus (Sturm, Gates, and Murphy, 1932) and tobacco mosaic virus (Hollaender and Duggar, 1936) required least amounts of energy for inactivation at the shortest wave lengths used: 2,380 and 2,250 Å, respectively. Rous sarcoma virus shows a minor peak in sensitivity between 2,500 and 2,700 Å, in addition to the maximum below 2,380 Å. *B. prodigiosus* (*Serratia marcescens*) is most sensitive to ultraviolet at 2,801 Å (Ehrisman and Noethling, 1932).

In general, the inactivation spectra referred to cannot be interpreted as corresponding exactly to the absorption spectra of certain specific substances within the microorganisms. However, there is considerable evidence that certain inactivation spectra are related to the effect of ultraviolet radiation on nucleoproteins (maximum absorption at 2,650 Å) or proteins (maximum absorption at 2,800 for proteins containing aromatic amino acids; at wave lengths below 2,650 Å for proteins without appreciable content of aromatic amino acids), depending on the case. Loofbourow (1948) and Giese (1945) have recently reviewed the theory of inactivation spectra and the experimental results.

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The purpose of this work was to ascertain whether two aspects of the same microorganism, namely the infectivity and hemagglutinating ability of the influenza virus, showed differences in sensitivity to ultraviolet radiation at different wave lengths. In preliminary experiments several batches of influenza virus, purified by means of Sharples supercentrifugation, were used. The same batch, purified by means of methanol precipitation, was used in the experiments reported in this paper. One batch of purified virus was used in order to make the results of different experiments more comparable and in order to reduce the intensity requirements and the effect of impurities.

MATERIALS AND METHODS

Virus. The starting material used in the preparation of the purified specimen consisted of 2,700 ml of freshly harvested PR-8-infected allantoic fluid. To this was added 675 ml of absolute synthetic methanol to make a final concentration of 20 per cent methanol. The addition of methanol was done stepwise, keeping the temperature of the allantoic fluid in the mixture between 4 and 6 C. The pH of the allantoic fluid was 7.4 at the start of methanol addition. The precipitated material was allowed to stand at 4 C for 1.5 hours and then was centrifuged at 2,000 rpm for 30 minutes in an International centrifuge, size 3. The supernatant was discarded. The precipitate was blended in a Waring blender with 135 ml of 0.3 molar phosphate buffer, pH 7.0. This was allowed to stand 1 hour at room temperature, then centrifuged in a no. 1 International centrifuge at 2,000 rpm for 10 minutes. The precipitate was discarded. The eluate was then centrifuged in a "quantity centrifuge" (ultra) at 20,000 rpm for 1 hour to sediment the virus. The pellets were dissolved in 0.1 molar phosphate buffer to make a concentration of approximately 5 times that of the original fluid.

Handling of the virus. The final experiments were performed over the course of 2 months. In 2 months the infectivity dropped from E.I. 50 (egg infectious, 50 per cent end point) of $10^{-6.1}$ to E.I. 50 of $10^{-4.75}$, whereas the hemagglutinating ability remained unchanged. The virus preparation was not frozen, because purified preparations were found to lose very considerable amounts of activity upon freezing. This held true for both the infectivity and the hemagglutinating ability. Before each experiment an aliquot of the source virus was centrifuged at 2,500 rpm for 1 hour at 4 C in order to remove the slight amount of flocculent precipitate that had formed in the source virus upon standing in the icebox. No demonstrable virus activity as measured by the hemagglutination test was discarded in the sediment. The supernatant was diluted 1:6 or 1:12 with 0.1 M phosphate buffer, pH 7, and distributed in 18-ml amounts into test tubes. The material was transported to the irradiation laboratory in a thermos jug containing ice. For irradiation one tube was taken out at a time, 15 ml being pipetted into the irradiation cell and the remainder being left standing on the desk for the duration of the irradiation. This was used as a control. In addition to these controls, special experiments were performed to rule out the possibility that part of the drop in titer shown by the irradiated material was related to other factors than irradiation. Specimens were stirred in the irradiation cell but protected against radiation. Aliquots removed after periods of time comparable to periods

used in irradiation experiments did not show decrease in the infectivity or the hemagglutinating titer.

In infectivity experiments 0.5-ml specimens were removed from the irradiation cell after various time intervals of irradiation and placed in small test tubes. At the end of a run at a certain wave length, all tubes were returned to the jug and a new tube was taken out for the next run. The tubes were protected against scattered radiation and light while standing on the desk. The infectivity experiments were all conducted at night at room temperature. Usually eggs were inoculated immediately upon returning to the virus laboratory, although on a few occasions there was a delay of several hours during which time the irradiated material and controls were kept in the dark at 4 C.

In the hemagglutination experiments the titrations were made while the irradiation was still in progress, since periods as long as 7.5 hours were required to inactivate the hemagglutinating ability of the influenza virus with monochromatic ultraviolet. Fifteen ml were used as the initial volume, and at various time intervals three specimens, 0.2 ml each, were removed in succession and their hemagglutination titers determined separately.

Definition of end points. The infectivity end point was determined as follows: Groups of 10- or 11-day-old embryonated eggs (3 to 5 per group) were inoculated with a 10^{-3} dilution of specimens irradiated for different periods of time. The dose of irradiation that resulted in a 50 per cent infectivity when the 10^{-3} dilution of irradiated material was inoculated was considered as the end point. Preliminary experiments were necessary in order to establish the approximate sensitivity ranges for different wave lengths. In final experiments, it was possible to group the specimens, taken at relatively short intervals, more closely around the end point. The end points were sharp: frequently all eggs were infected at the time point above, and no eggs were infected at the time point below. At other times the transition occurred in three stages: all infected, some infected, none infected. The time intervals varied from experiment to experiment. As an example, on June 6, 1949, with 2,652 A, specimens were collected in 30, 60, 120, 180, 300, 420, and 900 seconds. After 180 seconds of irradiation, all eggs inoculated were still positive, i.e., allantoic fluids collected from these eggs after a 48-hour period of incubation at 37 C and a 12-hour period of chilling at 4 C showed the hemagglutination phenomenon. After 300 seconds all eggs were negative. The end point dose was calculated by interpolation as follows:

$$\frac{50\% - \% \text{ of noninfected eggs at time point next below}}{\% \text{ of noninfected eggs next above} - \% \text{ of noninfected eggs next below}} = \frac{50\% - 0\%}{100\% - 0\%} = \frac{1}{2}$$

The dose delivered between the 180th and 300th second was multiplied by one-half and added to the cumulative dose that had been delivered in the first 180 seconds of irradiation; the sum was called the end point.

The assumption was made that the Bunsen-Roscoe law of reciprocity of time and intensity was operating in our experiments. This assumption was tested in one infectivity experiment. Full light from a mercury arc ("labarc") was used,

and it was found that doubling the distance of the irradiation cell from the source resulted in a fourfold increase in the time needed to reduce the infectivity of the influenza virus to the same extent. Since full light, shorter distances, and only 3 time points were used, this experiment can be considered only a gross check.

An experiment at 2,652 Å, making two runs at different intensities, tested reciprocity of time and intensity in the definition of doses for inactivation of the hemagglutinating ability. The intensities were related as 2:3, but doses required for the inactivation differed by only 15 per cent. The lower intensity required the larger final dose.

A linear relationship between the log survival ratio and time of irradiation (intensity being constant) has been reported for the inactivation of bacteria and viruses, and deviations occurring under certain circumstances have been pointed out.

Taylor *et al.* (1941), working with the equine encephalomyelitis virus, showed that when the inactivation of virus activity was conducted on a solution containing 0.20 mg of protein per ml, considerable departure from the straight line was present in the later phases of the reaction, whereas a solution containing 0.01 mg of protein per ml gave a straight-line relationship, and a 0.02 mg per ml solution gave a curve that showed only a slight deviation in the later phase of the reaction. They also found that suspensions of 10^5 *Serratia marcescens* per ml absorbed negligible amounts of 2,537 Å, and that the inactivation rate was independent of concentration. The stock solution of the influenza virus used in our experiments had a N concentration of 0.04 mg per ml. It was used in dilutions of 1:6 or 1:12. The stock solution also contained approximately 10^5 small gram-negative bacteria per ml that were identified as being *Alcaligenes faecalis*.

As was mentioned above, the infectivity titer of the virus preparation dropped during the 2-month period over which the experiments were conducted. For that reason absolute dose values from different experiments cannot be directly compared since they are based on somewhat different survival ratios. Relative values, obtained by normalizing the end point doses at wave length 2,803 for all the experiments, were used in comparing and summarizing the results of different experiments.

The *hemagglutination* phenomenon by the influenza virus was discovered independently by Hirst (1941) and McClelland and Hare (1941). Several methods for the determination of hemagglutination titers have been described. A modified Salk pattern test was used (Salk, 1944). Twofold dilutions of the virus suspension were made in 0.85 per cent NaCl solution, and 0.1 ml of 1 per cent chicken red blood cells in 0.85 per cent NaCl was added to each hemagglutination tube containing 0.2 ml of virus dilution. The tests were read in 1 hour at room temperature. The last tube showing definite (3+) hemagglutination was taken as the end point. The reciprocal of the final dilution of virus at the end point was called the hemagglutination titer. The three separate tests frequently gave the same end point; in other cases one of the three showed an end point one dilution lower or higher than the other two. In those cases, the titer shown by two tubes was taken as the end point.

Equipment and procedures for irradiation. The ultraviolet radiation used in these experiments was supplied by a 250-watt "uviarc" placed at the entrance slit of a large water-prism monochromator essentially similar to that of Harrison (1934). An aluminized spherical mirror of 8-inch diameter and 8-foot radius of curvature placed slantwise in a bowl of water provided a series of images of the source slit dispersed in the characteristic line spectrum of mercury. A framework bearing the exit slit and equipment for irradiation and radiometry was maneuvered into proper focus and alignment for the isolation of the several wave lengths used.

The irradiation cell was constructed of brass, later gold-plated. Quartz windows provided for entrance and exit of the monochromatic ultraviolet beam, and the cell was shaped to place as much as possible of the virus suspension directly within the beam. A magnetic stirring system kept the cell contents in motion.

The measurements of radiant intensity were made with a GL-935 phototube and a d.c. amplifier, and were corrected for varying spectral sensitivity according to the manufacturer's calibration, which in turn was checked using a thermopile and galvanometer. A recent calibration of the thermopile by the Eppley Laboratories gives the best indication of the absolute intensities involved in this work.

The measurements of the ultraviolet transmission by the influenza virus suspensions were made with a Beckman spectrophotometer, using 0.1 M phosphate buffer as a blank. The blank, tested against distilled water, showed no absorption at the wave lengths employed.

In the irradiation of suspensions radiation doses are often defined in terms of energy absorbed per unit volume or per organism—the latter when the organisms are in pure suspension in a nonabsorbing medium. When other ultraviolet-absorbing materials are present, their contribution to the total ultraviolet absorption and relevancy to the inactivation process being unknown, an inactivation spectrum in terms of energy absorbed is summed in with these extraneous absorption spectra. Hollaender and various co-workers have relieved this difficulty by irradiating dual suspensions, when one of the organisms involved (*E. coli*) had been previously irradiated in effectively pure suspension. The action spectrum for the viruses studied gains considerably in significance by the fact that on a relative basis the spectrum for *E. coli* is similar in pure and dual suspension.

The method that we have used to define and calculate radiation doses has the effect of reducing the suspension to a surface array of virus, irradiated by a known average intensity (see legend accompanying table 1). Any absorption in the suspension, whether relevant to the inactivation process or not, reduces this average intensity, but in a calculable manner. That is the reason for using such a definition: the average intensity to which the virus was actually exposed may be determined, whereas the absorption per organism in our suspensions could not. No method of defining dose can clearly separate the inactivating component of absorption within the virus from a noninactivating component, so that both intensity and absorption definitions are ambiguous in this respect.

In figure 1 is diagramed the arrangement of the equipment used in irradiation and radiometry. Phototube measurements were made in three positions. They

have been treated, as discussed in the explanation accompanying table 1, as simple measures of intensity on a collimated beam. Actually the beam diverged in the plane of the diagram by 1 part in 10 of the distance from the monochromator slit. There was some divergence perpendicular to this plane also, but by only about 1 part in 100 of the distance from the slit. Since irradiations are often made with divergent beams, the method that we have used in applying such a beam to volume irradiation may be of interest.

The beam width transverse to the slit (i.e., in the plane of the diagram) was exceeded by that of the irradiation cell windows and of the photoelectric surface in the two positions of measurement, before and behind the cell. Neglecting for the moment any variations in intensity parallel to the slit, it is seen that the phototube measurement is proportional to a total rate of energy flow into or out of the cell. For the absolute calibration the phototube was placed farther back behind the second slit shown in the diagram. This slit served to isolate a more nearly uniform center section of the beam, the transverse width of which was

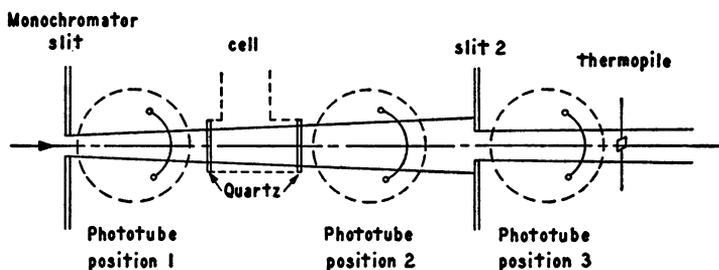


Figure 1. Diagram of the irradiation apparatus.

again fully intercepted by the photoelectric surface. Readings were made, alternating the phototube with the thermopile behind this second slit, varying the thermopile position for a better average intensity. Using the measured width of the beam at this position, the thermopile measurements were rendered into ergs per second per unit length parallel to the slit in calibrating the phototube. The phototube measurements before and behind the cell could now be put in terms of ergs per second by multiplying by the length of the cell windows parallel to the slit. Because of the monochromator astigmatism the intensity was quite uniform parallel to the slit; moreover, the cell windows and the photoelectric surface had nearly the same length in this direction.

The application of the absorption law to these energy rates within the cell in calculating an average is straightforward, as this law is based directly on rates of energy loss from a beam although often expressed in terms of intensities for a collimated beam. An arbitrary cross-sectional area of the beam may be chosen to render the measured energy rates into intensities. This arbitrary area is again used in calculating the beam volume within the cell, and thus drops out of the final expression for I_{av} . The slight beam divergence perpendicular to the plane of the diagram was taken into account in calculating I_v and I_d , but not in the distribution within the cell. The effect of this divergence could have been eliminated

with the other by limiting the beam at the monochromator slit so that it did not spread beyond the photoelectric surface or the cell windows in any direction.

RESULTS

Figure 2 shows the percentage of transmission of the virus specimens at the four wave lengths employed in the irradiation experiments. These curves were obtained on material used in infectivity experiments of June 6, 1949. More detailed transmission studies showed that below 2,400 Å the preparation was absorbing heavily (25 per cent transmission at 2,300 Å for the diluted 1:6 material). More detailed curves (10 to 15 points) did not show deviations exceeding 3 per

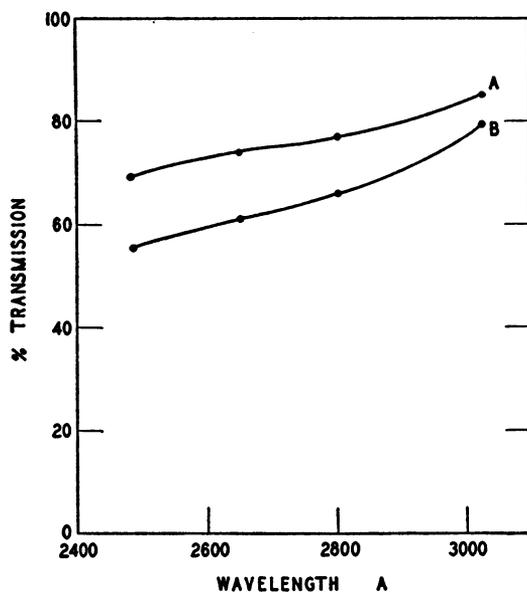


Figure 2. Transmission curves for the influenza A virus suspension in 0.1 M phosphate buffer. A, 1:12 dilution of stock suspension. B, 1:6 dilution of stock suspension.

cent from the ones reproduced in figure 2. The extent of nonviral absorption present in these curves is not known.

In the irradiation experiments wave lengths 2,487, 2,652, 2,803, and 3,023 Å were used. On several occasions all four wave lengths were used in the same experiment, thus assuring a fair degree of constancy in experimental conditions for different wave lengths. In addition, certain experiments were devoted to the comparison of two wave lengths. The average number of runs per wave length was three. As was mentioned above, infectivity end points were normalized against values obtained at 2,803 Å. Normalization was not attempted in hemagglutination experiments. Conclusions as to relative sensitivity of the hemagglutinating ability are based on the comparison of individual inactivation curves obtained with different wave lengths.

TABLE 1
 Protocol of a typical infectivity experiment; June 6, 1949; 2,652 and 2,803 Å

1	2	3	4	5	6	7	8	9	10
λ	Δt	I_o	I_d	ad	$\frac{I_o - I_d}{ad}$	$\frac{v}{V}$	I_{av}	Δ DOSE	TOTAL DOSE
λ	sec	$\frac{\text{ergs}}{\text{mm}^2 \times \text{sec}}$	$\frac{\text{ergs}}{\text{mm}^2 \times \text{sec}}$		$\frac{\text{ergs}}{\text{mm}^2 \times \text{sec}}$		$\frac{\text{ergs}}{\text{mm}^2 \times \text{sec}}$	$\frac{\text{ergs}}{\text{mm}^2}$	$\frac{\text{ergs}}{\text{mm}^2}$
2,652	30	5.1	1.0	1.63	2.5	0.440	1.11	33.2	33
	30					0.445	1.14	34.3	68
	30					0.471	1.18	35.6	103
	30					0.489	1.23	36.9	140
	60					0.508	1.28	76.6	217
	120					0.528	1.33	160	377
	120					0.550	1.38	165	540
2,803	480	4.4	1.17	1.32	2.44	0.574	1.44	690	1230
	30					0.440	1.08	32.3	32
	30					0.455	1.11	33.4	66
	30					0.471	1.15	34.6	100
	30					0.489	1.20	35.9	136
	60					0.508	1.24	74.5	210
	120					0.528	1.29	155	365
	120					0.550	1.35	160	525
	480					0.574	1.40	670	1195

The relative accuracy of these figures is 2 per cent. The first two columns show, respectively, the wave lengths and the time intervals between specimen withdrawals. The intensity just inside the front cell window (see figure 1) is shown in column 3. This figure is calculated from the phototube measurements in front of the irradiation cell by means of the following correction factors: (1) the absolute intensity calibration of the phototube at one reference wave length (2,652 Å), (2) the spectral sensitivity of the phototube relative to this wave length, (3) a slight drop in total beam energy with increasing distance from the monochromator prism, and (4) the loss through the front quartz window. In column 4 is shown a similar figure for the intensity just inside the back window of the cell, based on phototube measurements behind the cell. The distribution of the intensity I along the path of the beam through the cell may be represented by the absorption law:

$$I = I_0 e^{-ax}$$

where x is distance along the beam path. Substitution of I_d for I and d for x gives

$$\frac{I_d}{I_0} = e^{-ad}, \ln \frac{I_0}{I_d} = ad.$$

A mean intensity along the portion of the beam between the two windows is now found by integrating $I dx$ over the limits 0 to d and dividing by d ,

$$\bar{I} = \frac{1}{d} \int_0^d I_0 e^{-ax} dx = \frac{I_0 - I_d}{ad}$$

In the table ad and \bar{I} appear in columns 5 and 6. \bar{I} applies to the volume of the beam inside the cell and is now corrected by the ratio of the beam volume to that of the whole cell contents to give I_{av} , the average intensity to which a virus particle is subject in its motion through the entire stirred suspension. It is assumed that the motion is effectively random over any one irradiation period. The volume ratios and I_{av} appear in columns 7 and 8; column 9 shows the increments of dose between specimen withdrawals:

$$\Delta \text{dose} = I_{av} \Delta t$$

The total dose sustained by each specimen up to the time of its withdrawal is shown in the last column. We have not taken account of radiation possibly scattered out of the beam into other parts of the suspension. Reciprocity of intensity and time in forming dose products is assumed.

Table 1 shows the protocol of an experiment in which the infectivity was studied using 2,652 and 2,803 Å. This same experiment was referred to in connection with the discussion of infectivity end points. For 2,652 Å the end point fell between 180 and 300 seconds. The cumulative doses at these time points were 217 and 377 ergs/mm², respectively. As explained above: $377 - 217 = 160$ ergs/mm²; $160 \times \frac{1}{2} = 80$ ergs/mm²; $217 + 80 = 297$ ergs/mm². The end point for 2,803 Å, calculated in a similar fashion, was 695 ergs/mm². Figure 3 is a summary of our infectivity results.

Henle and Henle (1947) have demonstrated that, of the different properties of the influenza virus, the hemagglutinating ability is most resistant to inactivation by ultraviolet. Our experiments confirm the result that several hundred times more energy is needed to inactivate the hemagglutinating ability than to inactivate the infectivity. Figure 4 gives the inactivation curves obtained in a

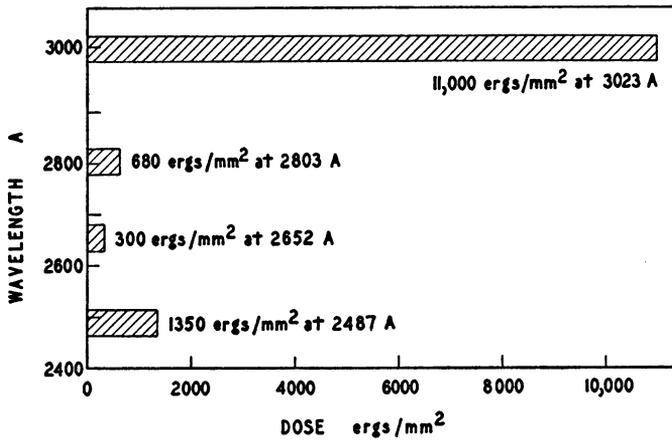


Figure 3. Resistance of the influenza A virus infectivity as a function of wave length.

36-hour continuous experiment. As can be seen, the greatest amount of energy is required for inactivation when 3,023 Å is used. A comparison of wave lengths 2,803 and 2,652 Å reveals that the hemagglutinating ability is more sensitive to the former. Even after 7 hours and 35 minutes of irradiation, the longest period employed, no effect on the hemagglutinating ability could be detected when 2,487 Å was used. In terms of energy delivered this means that the hemagglutinating ability is less sensitive to ultraviolet at 2,487 Å than at 2,803 Å. A relationship of sensitivity at 2,487 to that at 2,652 and 3,023 Å was not established because of the relative weakness of the 2,487 Å line in the mercury spectrum. These relationships were demonstrated repeatedly in similar experiments.

The manner in which the hemagglutinating ability remains undiminished in the first part of a run, later falling rather sharply, suggests that a multiple-hit process may be involved. Such sigmoid curves result for a collection of radiation-sensitive centers (or targets), absorbing radiation independently but organized into groups as regards activity, a group losing activity as a unit when some given number of its component centers have absorbed radiation. If only one center

per unit need be hit, the inactivation is logarithmic. For more than one per unit the inactivation becomes increasingly more sigmoid as the multiplicity increases. For comparison with the experimental points we have taken the family of multiple-hit curves, converted to the co-ordinates of this experiment, from those presented by Timoféeff-Ressovsky and Zimmer (1947), adjusting the dose scale for best fit with regard to multiplicity. Three such fitted curves may be seen in figure 4.

At 2,803 Å one run fitted a 5- or 6-hit process; another with fewer points appeared to lie between 6 and 10 hits. At 2,652 Å three sets of points were available, those in figure 4 suggesting a 35- to 45-hit process, whereas the two runs previously mentioned as a reciprocity check indicate 20 to 30 hits for the higher intensity and 50 to 100 for the lower. At 3,023 Å a multiplicity of at least 100

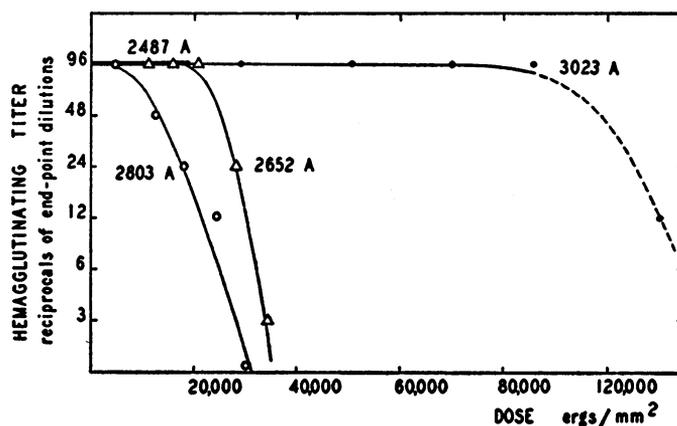


Figure 4. Inactivation curves for the hemagglutinating ability of the influenza A virus, experiment of June 18, 1949. The heavy line represents wave length 2,487 Å, for which the actual points are not shown. The three curves are theoretical fits from the family of multiple-hit curves: a 5-hit curve through the 2,803 Å points, a 40-hit for 2,652 Å, and a 100-hit for 3,023 Å.

seems indicated by the last two points shown in figure 4. Greater sensitivity to ultraviolet does appear to be associated with lower multiplicity. The variation of multiplicity with wave length and intensity is rather difficult to harmonize with the theory, even admitting the poor sensitivity of the data to the fitting process. Hemagglutination inactivation apparently involves more than a simple irreversible inactivation after some given number of targets have been hit.

No change in the appearance of the influenza virus particles could be observed under the electron microscope after exposure of the purified virus to energies that completely inactivated the infectivity (figure 5, A and B) or the hemagglutinating ability (figure 5, C and D). The specimens used represent materials from the infectivity experiment at 2,652 Å of June 6, 1949, and the hemagglutination experiment at 2,803 Å of June 18, 1949, respectively. These two experiments have been discussed in detail above. This negative result is in line

with Hamre's report (1949) that electron micrographs of influenza A virus, PR-8 strain, the infectivity of which had been inactivated by ultrasonic waves, were not revealing.

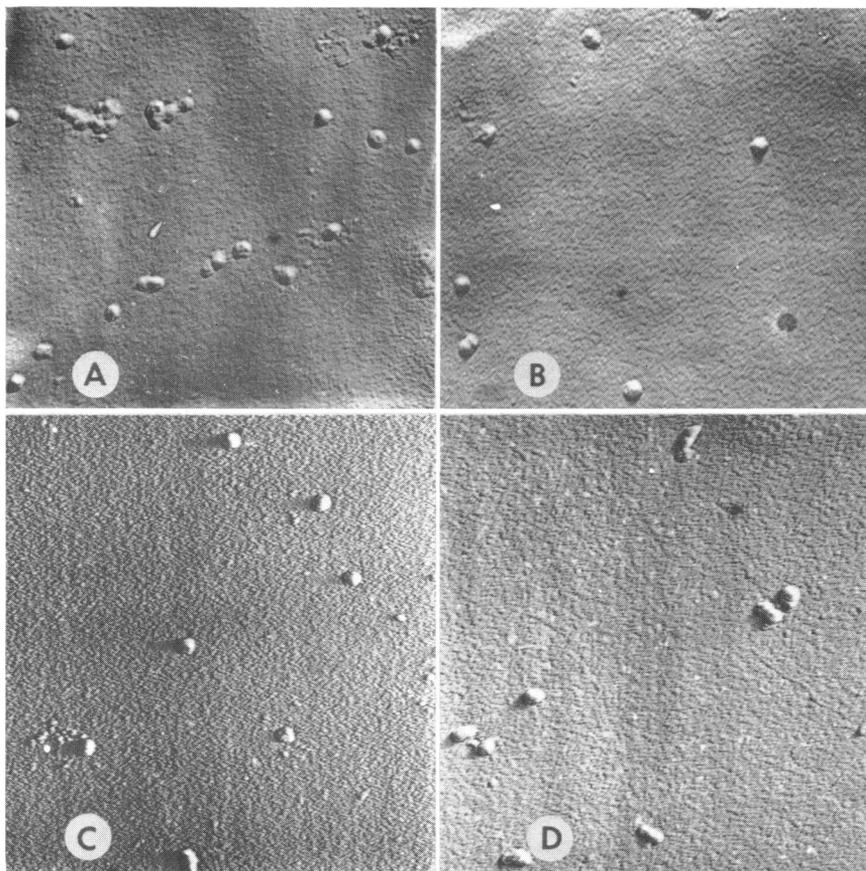


Figure 5. Influenza A virus in 0.1 M phosphate buffer. Pictures taken with electron microscope, magnification 15,400; chromium plated. A, control. B, infectivity inactivated. C, control. D, hemagglutinating ability inactivated.

DISCUSSION

Both the infectivity and the hemagglutinating ability of the influenza virus show a maximum sensitivity to ultraviolet within the span of the four wave lengths used. The infectivity is more sensitive to radiation at wave length 2,652 A than at 2,803 A, but the hemagglutinating ability conversely shows greater sensitivity at 2,803 A than at 2,652 A. Comparison of the results of our four points for infectivity with the experiments of Hollaender shows general agreement in the dependence of sensitivity upon wave length. This similarity is particularly significant in view of the experimental differences: in assay, in method of defining and measuring radiation doses, and very markedly in the

absorption spectra of the media irradiated. Thus our result constitutes additional evidence that infectivity of the influenza virus is related to a substance in the virus having an inactivation peak at a wave length at which nucleic acids show an absorption peak.

The hemagglutinating ability of the same influenza virus preparation indicates a different peak in sensitivity, suggesting that a different substance is responsible for this property of the virus. The indication is that the peak is at longer wave lengths; the result is not in conflict with the inclusion of proteins with aromatic constituents in this latter substance.

Should it be possible to show that the immunizing ability of the influenza or other viruses has an inactivation spectrum different from that of infectivity and toxicity, it might prove advantageous to apply monochromatic ultraviolet radiation to vaccine production, using wave lengths that are most effective against the latter properties of the virus, sparing the immunizing ability.

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SUMMARY

The infectivity of influenza virus type A, strain PR-8, is more sensitive to monochromatic ultraviolet at 2,652 Å than at 2,803 Å; the sensitivity of the hemagglutinating ability of the same virus, though several hundred times less over-all, is greater at 2,803 Å than at 2,652 Å.

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