THE INACTIVATING EFFECT OF MONOCHROMATIC ULTRAVIOLET RADIATION ON INFLUENZA VIRUS

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Received for publication May 18, 1944

The effectiveness of ultraviolet radiation in the inactivation of biological materials has made it valuable in the study of bacteria, fungi and viruses. The use of ultraviolet in the possible control of air-borne contagion has made it a promising practical tool (review by Hollaender, 1943). A study of the relative effectiveness of the different wavelengths of the spectrum between 2000 and 3000A may give us information in regard to the chemical structure of the irradiated substances.

This report will describe the efficiency of eight wavelengths between 2180A and 2937A in the inactivation of influenza virus A and compare it with the inactivation spectrum of *Escherichia coli* and of a number of other viruses and virus-like agents.

EXPERIMENTAL TECHNIQUE FOR PREPARATION AND TESTING OF VIRUS MATERIAL

Allantoic fluid from chick embryos inoculated into the allantoic sac with influenza Virus A was used in all experiments. In earlier work the infected fluid was harvested and used without modification except for centrifugation (10 min.) at 1000 rpm. In later work the virus was adsorbed on chick-embryo blood cells, eluted and concentrated 5-fold according to the method of Francis and Salk (1942).

Inoculation of embryos: 10–11 day eggs incubated at 36.5–37.0 C were inoculated into the allantoic sac with a syringe with .05 ml of a bacteriologically sterile Berkefeld N filtered 1:100 suspension of mouse lung from mice inoculated 3–4 days previously with the PR 8 strain of mouse-adapted influenza virus. After 48 hours' further incubation the allantoic fluid was removed, sealed in 1 ml amounts in glass ampoules, rapidly frozen and stored at −70 C.

Test procedure: An ampoule of frozen virus was thawed in a 37 C water bath and diluted 1:50 with 1% broth—normal saline (phosphate buffered, pH 7.6). The suspension was divided in two equal portions; one portion served as control and the other was irradiated; both suspensions were handled in the same way except the control was protected against radiation. During irradiation, aliquots were removed at varying time intervals from the irradiated portion and stored at 4 C. After irradiation was completed two ten-fold dilutions with 1% broth saline were made of each aliquot and the control virus suspension; thus, for each sample there were three dilutions, 1:50, 1:500 and 1:5000. Groups of 6 white Swiss mice weighing 12–15 gm were inoculated intranasally under light ether anesthesia with 0.05 ml of each dilution. Deaths were recorded daily for ten days and surviving mice were autopsied at the expiration of the period.

Consolidation of the lung was graded +, ++, +++, or ++++ according to the usual method, + indicating less than 25% involvement, ++ less than 50%, +++ less than 75% and ++++ more than 75%.

A final score was given each group by the following method. A death was given a grade of 5. The days subsequent to inoculation were numbered in the reverse order, the tenth day, number two, the ninth, number 3, and so on. Thus, if a mouse died on the tenth day its final score was 5 × 2 or 10. A death on the 7th day (No. 5) would be graded 5 × 5 or 25. Surviving mice were graded, 0, 1, 2, 3 or 4 according to the amount of lung consolidation found. The scores of each group were then totaled.

A part of the protocol of one irradiation experiment is given in table 1 to illustrate the method of scoring used.

PREPARATION AND TESTING OF BACTERIA

A stock culture of *E. coli* was incubated at 37 C on beef-extract peptone agar slants for 48 hours; the surface growth washed off with physiological salt solution, shaken, filtered through absorbent cotton, centrifuged, resuspended in salt solution and refiltered. The bacteria were irradiated in the virus suspension in a dilution of 10^7 organisms/ml. The suspension containing the bacteria was used only for testing the survival of the bacteria. Samples of 1/10 ml were removed before and during the process of irradiation, diluted in physiological salt solution and plated out in nutrient agar. At least three plates were poured for each dilution and as many as three dilutions were made for each exposure. All colonies were counted on plates having 30 to 300 colonies per plate after incubation for 48 hours at 37 C. All data given in the tables are averages of at least 3 plates.

PHYSICAL TECHNIQUE

Radiation from a medium-pressure water-cooled quartz capillary mercury vapor lamp of the Daniels-Heidt type was concentrated on the entrance slit of a quartz monochromator. The emerging monochromatic beam was concentrated on a standardized thermopile or the window of an exposure cell which held about 10 ml of the suspension. The contents of the cell were stirred thoroughly to insure uniform irradiation of each particle. The technique used in this experiment is very similar to the one described in previous publications (Hollaender and Claus, 1936, and Hollaender and Duggar, 1936). The wavelengths used were 2180, 2280, 2380, 2480, 2537, 2650, 2805 and 2967A. The purity of the lines was fairly high since narrow slits were used and the energy used in inactivation of the virus was fairly low. In the early part of this work a water bath to keep the radiation cell at 10 to 15 C was used. In later experiments the exposure cell was not water-cooled since no difference in the irradiation effect between 15 and 25 C could be recognized in the relatively short time of exposure. The exposure cell contained about 10 ml, and 0.8 ml was removed for virus titration at intervals through a side arm of the cell without interfering with the process of irradiation. The periodic reduction in content of the cell was considered in calculation of the energy received. For details of calculation see table 1.

The spectral transmissions of the allantoic fluid in dilution of 1/100 and 1/500 in 1% broth are given in fig. 1. The relatively high absorption by this solution of the shorter wavelengths might protect the virus against the action of these wavelengths. However, this is overcome to a very high degree by the thorough stirring employed in these experiments. The irradiation of *E. coli* in the virus suspensions gave a wavelength dependence curve very similar to the one given when this organism was irradiated in a salt solution non-absorbent for the wavelengths used (Hollaender and Claus, 1936), although the absolute energy to produce a certain effect in salt solution was lower than the one needed in dilute allantoic fluid.

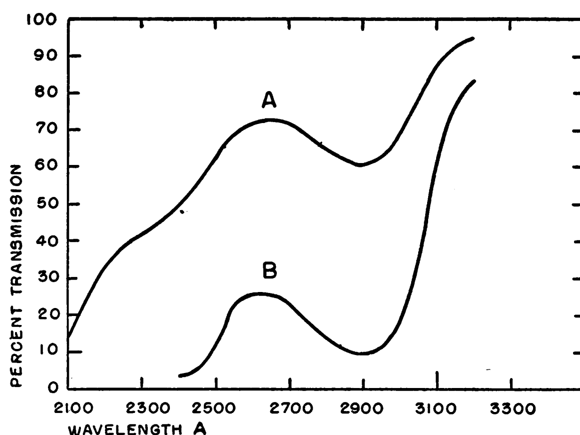

FIG. 1. Transmission curves (ultraviolet radiation) for allantoic fluid. A. Normal allantoic fluid diluted 1:50 with normal meat broth and then 1:10 with physiological salt solution. B. Normal allantoic fluid diluted 1:50 with normal meat broth and 1:1 with physiological salt solution.

RESULTS

The results of a typical virus experiment are given in table 1. Such data have been obtained for all of the 8 wavelengths used. The tests were repeated for each wavelength 3 to 4 times with the exception of wavelength 2180. Variation of sensitivity of separate batches of virus material can be seen in fig. 2 where we plotted the survival ratio against incident energy at wavelength 2650A from data obtained over a period of two years.

Data for 25, 50, 75 and 90% inactivation of the virus are given in table 3. A wavelength graph for 10% survival at 8 wavelengths is given in fig. 3. This figure also shows a graph for the inactivation of *E. coli* in the virus suspension but tested in separate experiments. The values given are for incident energies. Amount of radiation absorbed can be estimated only indirectly. The energy values for incident and absorbed energy to produce a certain percentage of killing in *E. coli* have been well established. Since both agents were irradiated in the same suspension one can estimate the sensitivity of the virus from the sensitivity of *E. coli*. The graph as given in fig. 2 demonstrates the relative sensitivity of these two agents. Such data are fairly reliable since the screening action of suspended matter will protect the virus as well as bacteria in the same suspen-

TABLE 1

Method of scoring

Experiment V21, 4/8/42, 2650A

INOCULUM	EXPOSURE	DILUTION	8 4/10	7 4/11	6 4/12	5 4/13	4 4/14	3 4/15	2 4/16	(a) (b)	LUNG LESIONS IN SURVIVORS	GROUP SCORES	TOTAL SCORE
	minutes												
First portion of virus irradiated	5	10^{-2}			5	1						175	
		10^{-3}				2	2	1	1	(c)		115	
		10^{-4}						1			3, 3, 3, 2, 2	28	
		10^{-5}									0, 0, 0, 0, 0, 0	0	318
Control virus not irradiated	0	10^{-2}	1	3	2							205	
		10^{-3}	1		2	2	1					170	
		10^{-4}					1	3	1		2	77	
		10^{-5}									1, 1, 0, 0, 0, 0	2	454

(a) Number assigned to each day after inoculation in reverse order for purpose of scoring.
(b) Date.
(c) Deaths of animals.

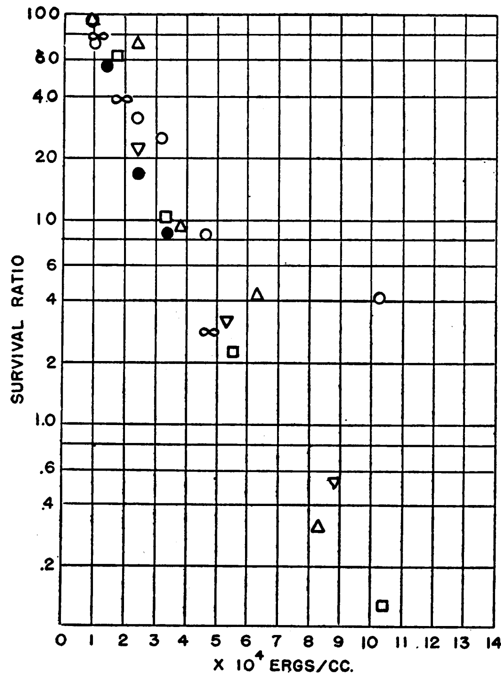

FIG. 2. Graph of survival at 2650A against incident energy. Each type of sign indicating tests made with one batch of material. Experiments were spread over a two year period.

TABLE 2

Protocol of a typical experiment

Experiment V21, 4/8/42, 2650A

Exposure cell content at beginning of test = 10 ml.
Galvanometer deflection 7 cm at 46.7 ergs/cm deflection.
Typical calculation for exposure 1 (5 min):

$$\frac{46.7 \text{ (ergs/cm deflection)} \times 7 \text{ (cm. deflection)} \times 300 \text{ (time of exposure in seconds)}}{10 \text{ (ml in exposure cell)}} = 9.8 \times 10^3 \text{ ergs/ml}$$

Sample of 8/10 ml removed for titration.
Calculation for exposure 2 (5 min):

$$\frac{46.7 \times 7 \times 300}{9.2} = 10.6 \times 10^3 \text{ ergs/ml}$$

Add this energy value to the one received in Exposure #1 $10.6 \times 10^3 + 9.8 \times 10^3 = 20.4 \times 10^3$/ergs/ml.

This test included 5 exposures. A separate control was made at the same time handling the material in identical manner as for exposure with the exception of protecting it against the radiation.

RUN NUMBER	TIME OF EXPOSURE	INCIDENT ENERGY	VIRUS TITRATION	SURVIVAL
	min	*ergs/ml*		%
1	5	9.8×10^3	318	70
2	10	20.4×10^3	141	31.2
3	15	32.0×10^3	90	19.6
4	30	44.9×10^3	38	8.4
5	51	95.5×10^3	26	5.7

Graphs of energy/ml against titration values were plotted for each experiment and the figures given in table 3 are taken from such graphs.

Separate tests were made for the determination of the sensitivity of *E. coli* in the virus suspension. These tests were conducted in an identical manner with the ones described for virus testing only. The number of exposures for the bacterial tests was increased to 8 or 10 since the titration of the bacteria was a very simple procedure.

TABLE 3

Energies for the inactivation of influenza virus and E. coli

WAVELENGTH IN A	ENERGY (ERGS)/ML FOR INACTIVATION OF VIRUS				ENERGY OF INACTIVATION FOR E. COLI
	25%	50%	75%	90%	90%
2180	10	34	75 (?)	75	32
2280	34	52	74	110	40
2380	20	40	60	78	52
2480	18	28	36	48	38
2537	8	16	26	38	30
2650	6 (?)	16	24	34	22
2805	10 (?)	18	26	40	34
2967	120	180	280	350	320

The values marked (?) are questionable. Multiply all values by 10^3.

sion. The only possible exception would be that if the virus particle is surrounded by extraneous matter which is closely attached to the virus particle this comparison might be erroneous. However, it appears that if such matter is present it can be only in a very small amount and is probably readily penetrated by ultraviolet radiation. The relatively high sensitivity of influenza virus at 2537 has been reported by several investigators but no report has shown wavelength dependence of inactivation with ultraviolet (Wells and Brown, 1936, Edwards, Lush and Bourdillon, 1944).

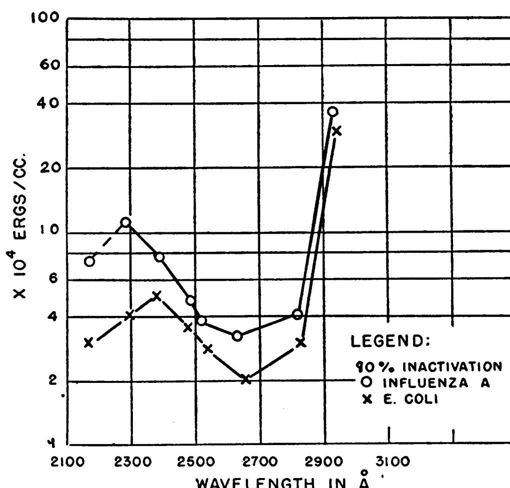

FIG. 3. Graph of the energy to produce 90% inactivation against the wavelength; (O) influenza virus, (X) *E. coli* irradiated in virus suspension.

DISCUSSION

Data are now available for the wavelength dependence of inactivation for 5 sub-microscopic agents: Tobacco mosaic (Hollaender and Duggar, 1936); chicken tumor (Sturm, Gates and Murphy, 1932); vaccine virus (Rivers and Gates, 1928); bacteriophage (staph.) (Gates, 1934), and influenza virus. The data for chicken tumor virus were only obtained down to 2380A and they have been extrapolated to 2200A.

The chicken tumor, vaccine virus and bacteriophage were irradiated in thin layers usually spread over a nutrient agar. Data for tobacco mosaic and influenza virus were obtained by irradiating them in liquid suspensions which had little absorption for ultraviolet. Both methods showed theoretically similar results and the data can be compared readily.

If we take the energy necessary to inactivate at the wavelength which is most effective as 100 and divide by the energies of other wavelengths and plot these results for the eight wavelengths we tested, we obtain the graph shown in fig. 4. On the basis of these findings virus and virus-like agents can be divided into 2 groups; one with very high sensitivity at wavelengths shorter than 2300A and a slight maximum of sensitivity at 2600A. To this group belong the viruses of tobacco mosaic and chicken tumor 1. Both of these viruses have been reported to contain, besides protein, ribose nucleic acid (Bawden and Pirie,

1937, Loring, 1939, Claude, 1938). In the second group we have vaccine virus, influenza virus and bacteriophage. All these agents have very high sensitivity at wavelength 2650 and decreased sensitivity at shorter and longer wavelengths. Vaccine virus (Hoagland, Lavin, Smadel and Rivers, 1940) and influenza virus

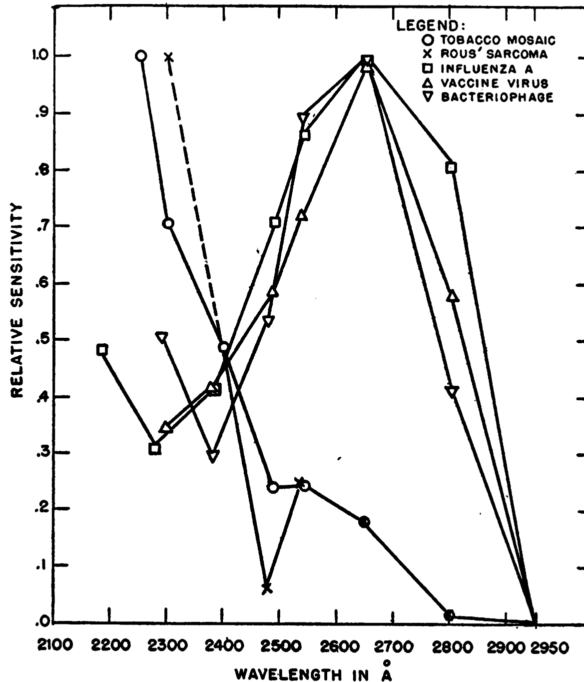

FIG. 4. Plot of the relative sensitivity against the wavelength for tobacco mosaic, chicken tumor 1, influenza A, vaccine virus and bacteriophage taking the energy at the wavelength which is most effective as 1 and dividing by the less effective energies. (For references see text.)

(Taylor, Sharp, Beard, Beard, Dingle and Feller, 1943) have been reported to contain mainly desoxyribose nucleic acid. However, it is important to keep in mind that in the present status of study of the chemical composition of these last two viruses one cannot be certain whether the material is actually purified to a high enough degree to permit a conclusion as to their composition (Stanley and Knight, 1944, Chambers, Henle, Lauffer and Anderson, 1943).

It is important to point out that the inactivation spectra of bacteria, fungi and yeasts show a maximum of sensitivity at 2650A. This wavelength is also most efficient for the production of mutations in fungi, maize, and liverwort. (For review see Hollaender and Emmons, 1941.)

It is quite possible that the high sensitivity of many agents at about 2600A is based on the important function desoxyribose nucleic acid plays in biological activities. The relatively high sensitivity of tobacco mosaic and chicken tumor viruses may be caused by the high absorption of the proteins at wavelengths around 2300A. A decision in regard to the above points could only be arrived at by further experiments.

SUMMARY

The sensitivity of influenza virus to monochromatic ultraviolet radiation was determined and compared with the sensitivity of *Escherichia coli* in the same virus suspension.

The wavelength dependence of inactivation of five viruses or virus-like agents is compared (influenza, vaccine, bacteriophage, chicken tumor and tobacco mosaic) and the significance of these findings discussed.

REFERENCES

BAWDEN, J. C., AND PIRIE, N. W. 1937 The isolation and some properties of liquid crystalline substances from solanaceous plants with three strains of tobacco mosaic virus. Proc. Roy. Soc. B., **123**, 274–319.

CHAMBERS, L. A., HENLE, W., LAUFFER, M. A., AND ANDERSON, T. F. 1943 Studies on the nature of the virus of influenza. J. Exptl. Med., **77**, 265–276.

CLAUDE, A. 1938 Concentration and purification of chicken tumor I agent. Science, **87**, 467–468.

EDWARDS, D. G., LUSH, DORA, AND BOURDILLON, R. B. 1944 Studies on air-borne virus infections. II. The killing of virus aerosols by ultraviolet radiation. J. Hyg., **43**, 11–75.

FRANCIS, T., AND SALK, J. E. 1942 A simplified procedure for the concentration and purification of influenza virus. Science, **96**, 499.

GATES, F. L. 1934 Results of irradiating *Staphylococcus aureus* bacteriophage with monochromatic ultraviolet light. J. Exptl. Med., **60**, 179–188.

HOAGLAND, C. L., LAVIN, G. I., SMADEL, J. E., AND RIVERS, T. M. 1940 Constitution of elementary bodies of vaccinia. II. Properties of nucleic acid obtained from vaccine virus. J. Exptl. Med., **72**, 685–696.

HOLLAENDER, A. 1943 Ultraviolet irradiation as a means of disinfection of air. Am. J. Pub. Health, **33**, 980–984.

HOLLAENDER, A., AND CLAUS, W. D. 1936 The bactericidal effect of ultraviolet radiation on *Escherichia coli* in liquid suspension. J. Gen. Physiol., **5**, 753–765.

HOLLAENDER, A., AND DUGGAR, B. M. 1936 Irradiation of plant viruses and of microorganisms with monochromatic light. III. Resistance of the virus of typical tobacco mosaic and *Escherichia coli* to radiation from λ 3000A to λ 2250A. Proc. Natl. Acad. Sci. U. S., **22**, 19–24.

HOLLAENDER, A., AND EMMONS, C. W. 1941 Wavelength dependence of mutation production in the ultraviolet with special emphasis on fungi. Cold Spring Harbor Symposia Quant. Biol., **9**, 179–186.

LORING, H. S. 1939 Properties and hydrolytic products of nucleic acid from tobacco mosaic. J. Biol. Chem., **130**, 251–258.

RIVERS, T. M., AND GATES, F. L. 1928 Ultraviolet light and vaccine virus. II. The effect of monochromatic ultraviolet light upon vaccine virus. J. Exptl. Med., **47**, 45–49.

STANLEY, W. M., AND KNIGHT, C. A. 1944 An evaluation of methods for the concentration and purification of influenza virus. J. Exptl. Med., **79**, 255–300.

STURM, E., GATES, F. L., AND MURPHY, J. B. 1932 Properties of the causative agent of a chicken tumor. II. The inactivation of the tumor-producing agent by monochromatic ultraviolet light. J. Exptl. Med., **55**, 441–444..

TAYLOR, A. R., SHARP, D. G., BEARD, DOROTHY, BEARD, J. W., DINGLE, JOHN H., AND FELLER, A. E. 1943 Isolation and characterization of influenza A virus (P.R. 8 Strain). J. Immunology, **47**, 261–281.

WELLS, W. F., AND BROWN, H. W. 1936 Recovery of influenza virus suspended in air and its destruction by ultraviolet radiation. Am. J. Hyg., **24**, 407–413.