STUDIES ON AIR-BORNE VIRUS INFECTIONS

II. THE KILLING OF VIRUS AEROSOLS BY ULTRA-VIOLET RADIATION

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(With 1 Figure in the Text)

The use of ultra-violet radiation for disinfecting air appears likely in future to become of considerable practical importance. Under laboratory conditions its effectiveness against sprays of bacterial cultures was demonstrated by Wells & Wells (1936) and confirmed more recently by others (Andrewes *et al.* 1940). In practical trials it has been shown of value in reducing cross-infection in children's hospitals, schools and operating theatres, etc. (Wells, 1940; Wells, Wells & Wilder, 1942; Hart, 1940). Measles, mumps and chickenpox are among the infections whose spread appears to be diminished (McKhann, Steeger & Long, 1938; Greene, Barenberg & Greenberg, 1941; Wells *et al.* 1942). But the only laboratory investigations so far reported of the action of ultra-violet radiation on virus particles dispersed in the air have been those of Wells and his associates, who showed that aerosols of influenza virus could be rendered non-infective for ferrets and mice (Wells & Brown, 1936; Wells & Henle, 1941). It is the purpose of this paper to describe quantitative studies carried out early in 1940 of the killing effect of ultra-violet radiation upon aerosols of three viruses, influenza A, vaccinia and herpes simplex.

Technical methods

The technique and apparatus employed were a modification of those elaborated for investigating virus aerosols and described previously (Edward, Elford & Laidlaw, 1943). In the experiments with influenza virus 5% suspensions of mouse lungs infected with the P.R.8 strain were prepared in a mixture of equal parts of physiological saline and nutrient broth. Suspensions of vaccinia virus were obtained by emulsifying, each in 5 c.c. of nutrient broth, chick chorio-allantoic membranes that had been inoculated 3 days previously with a dermal strain of virus. The H.F. strain of herpes simplex virus, adapted to the egg by Burnet, Lush & Jackson (1939), was chosen for the experiments with that virus, and suspensions prepared in the same manner as those of vaccinia, except that the membranes were harvested 2 days after inoculation.

Atomization of the suspensions was effected with an Aerograph Pencil Spray; the aerosols were collected, and allowed to stand for 15 min. before use in an aspirator jar over a saturated solution of calcium chloride (giving an estimated water-vapour pressure at 18° C. of 5·4 mm. Hg = 35% rel. humidity). In the experiments with vaccinia and herpes viruses sampling of the aerosols was carried out by filling pots of 225 c.c. capacity each containing 2·25 c.c. of nutrient broth. The virus particles were deposited in the broth by centrifugation at 2000 r.p.m. for 30 min. Their number was estimated by inoculating the chorio-allantoic membranes of 12-day White Leghorn eggs with 0·05 c.c. of dilutions of the broth according to the technique of Burnet (1936). Counts of the

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resulting lesions were made after 2 days' incubation and the average taken for three membranes inoculated with each dilution. The infectivity of aerosols of influenza virus was estimated by noting the lesions produced in mice which were exposed to the aerosols in glass vessels for chosen periods of time.

The set-up of the apparatus for the tests with vaccinia and herpes viruses is shown in Fig. 1. For influenza virus the two centrifuge pots were omitted, the one distal to the irradiation vessel being replaced by the vessel for exposing mice. In the earlier experiments, including those with influenza virus, the flowmeter was not included.

Irradiation vessels

Two types of vessel were constructed for exposing aerosols to the action of ultra-violet radiation.

(A) For the first two experiments with influenza virus an annular silica cylinder was used, length 19·5 cm., mean radius 3·08 cm., distance between inner and outer walls 0·9 cm., and volume 343 c.c. The aerosol was led into it through a short straight glass tube (2 mm. bore) ending half-way along the cylinder and so giving a jet of air which reached the far end, spread out and then streamed back relatively slowly to the exit tube at the rear end of the cylinder. This vessel had the advantage of not retaining many particles, but it necessarily contained areas of relative stagnation which made accurate estimation of exposure time impossible. However, if a uniform flow throughout the vessel were assumed, a maximum exposure time could be easily calculated, and it is legitimate to infer that the major part of the escaping aerosol could not have had more than this maximum exposure.

(B) For later tests, including all those with vaccinia and herpes viruses, a silica spiral was used. It was made up of a tube 12·53 m. long, 3·2 mm. bore and 100 c.c. volume, twisted into a spiral of 4·75 cm. mean radius with 42 turns closely wound together so that the over-all length was 20 cm. The exposure time could be regulated by varying the number of turns of the spiral exposed to the radiation, the rest being screened by impervious black paper. The experiments showed that a varying degree of deposition of virus particles occurred on the walls of the tube. However, since the concentrations of aerosols that had passed through the tube, firstly with the lamp on and secondly with the lamp off, were compared, the retention of virus in the tube should not cause any appreciable error in assessing our results. After each experiment the spiral was cleaned with caustic soda solution followed by water, alcohol and ether.

Ultra-violet lamps

Two types of low-pressure mercury-vapour 'germicidal' lamps were used, both giving a very high proportion of their radiation at wave-length 2537 A. One was a Hanovia lamp in the form of a 12 in. straight silica tube with a silica jacket containing an aqueous solution which filtered out very short wave-lengths and thus practically prevented any ozone formation. It was fed from a transformer (about 400 V. running and 2000 V. starting current) taking approximately 15 W. in the primary circuit. The other was a G.E.C. 'Sterilamp' supplied by British Thomson Houston, Ltd., and made in U.S.A. It incorporated a tube 18 in. by 1 in. of special ultra-violet transmitting glass, and took

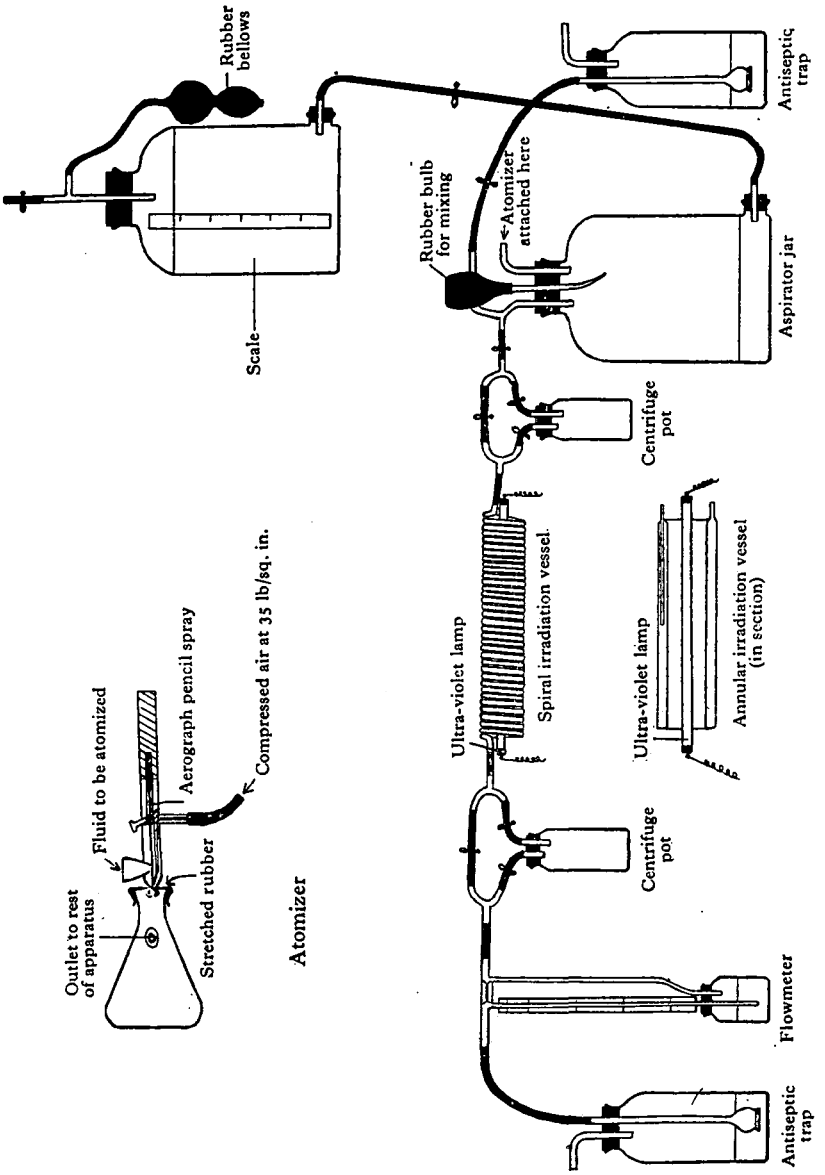

Fig. 1.

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about 18 W. at 210 V. 50 cycles (nominal 15 W.). The lamp is stated to give an ultra-violet output of 15–20 μW. per sq. cm. at 1 m. distance. Either lamp was mounted axially in the centre of the annular cylinder or spiral. The lamps were switched on about 5 min. before a test began, in order to ensure adequate warming up.

Results

The results of the experiments are summarized in the tables. Table 1 shows that radiation from both lamps was effective in killing aerosols of influenza virus exposed for 6 sec. or more. In only one experiment (no. 3) did a mouse that had inhaled irradiated aerosol show a lung lesion which from its appearance might have been a small influenzal lesion. The control mice were exposed to aerosols that had passed through the irradiation vessel with the lamp off. This part of the experiment was performed last so that the aerosol had aged more and was therefore slightly less infective. The tests suggest that ultra-violet radiation has effected at least a 99% kill, if not a complete kill. This opinion is based on experience gained in the titration of influenza virus aerosols.

Table 1. *Effect of ultra-violet radiation on aerosols of influenza virus*

				Mice exposed to irradiated aerosol		Control mice	
No. of exp.	Lamp	Irradiation vessel	Duration in sec. of exposure to ultra-violet radiation	Duration of exposure to aerosol in min.	Degree of infection	Duration of exposure to aerosol in min.	Degree of infection
1	Hanovia	Cylinder	Max. 60	35	0, 0, 0, 0, 0, 0	15*	5, 5, 5, 5, 5, 3
2	,,	,,	Max. 6	20	0, 0, 0, 0, 0, 0	1	5, 3, 3
3	,,	Spiral	8	25	?1, 0, 0, 0, 0, 0	1	3, 3, 2
4	'Sterilamp'	,,	6	30	0, 0, 0, 0, 0, 0	15	5, 5, 5, 3, 3, 3

* Chamber in which control mice were exposed was only half-filled with aerosol.

Resulting infection of mice is expressed in terms of a numerical index. Thus 5 represents death with influenzal consolidation of the lungs. Among survivors consolidation affecting three-quarters of the lungs is represented by 3, half by 2 and a quarter or less by 1.

Table 2. *Effect of ultra-violet radiation on aerosols of vaccinia virus*

All these experiments were carried out with the 'Sterilamp' and spiral irradiation vessel.

		No. of viable virus particles found per c.c. of aerosol		
			After passing through spiral	
No. of exp.	Duration in seconds of exposure to ultra-violet radiation	Before entering spiral	Lamp off	Lamp on
1	5	98·6	40–100	0
2	2·4	72	11·7	0
3	1·0	22	22	0
4	0·5	50	28	1·2
5	0·5	190	95	0·1

Tests with vaccinia were more detailed (Table 2). When the exposure time was 1 sec. or more, no viable virus was recovered from 15 c.c. of irradiated aerosol, whereas there were from 175 to 600 virus particles in the same volume of unirradiated aerosol. Thus it appeared that exposure of the aerosol to irradiation for as short a time as 1 sec. produced effective sterilization. A kill that was still probably more than 90% was obtained when the exposure time was reduced to 0·5 sec.

The results with herpes virus appeared to show a complete kill for exposures of 1 and 2·4 sec., and a partial kill for an exposure of 0·5 sec. However, the figures were irregular, owing apparently to some unknown source of error in estimating the number of virus particles, and hence these results can only be regarded as provisional.

SUMMARY AND CONCLUSIONS

The experiments show that rapid and effective sterilization of atmospheres containing atomized particles of influenza and vaccinia viruses, and probably also of herpes simplex virus, can be obtained by ultra-violet radiation of wave-length 2537 A. At least 99% and probably more of an aerosol of influenza virus was killed by exposure for 6 sec. at a distance of 2 cm. from either a Hanovia lamp or a G.E.C. 'Sterilamp'. These findings confirm the work of Wells and his associates who first demonstrated the susceptibility of this virus to ultra-violet radiation (Wells & Brown, 1936; Wells & Henle, 1941). At the same distance the 'Sterilamp' produced more than a 99% kill of vaccinia virus with an exposure of 1 sec. and about a 90% kill in 0·5 sec. The experiments with herpes were less satisfactory but suggested a similar sensitivity. These results support the view that 'germicidal' lamps are likely to be useful in reducing the infectivity of air contaminated with particles from persons suffering from virus infections of the respiratory tract.

We wish to acknowledge our indebtedness to the late Sir Patrick Laidlaw who, although his name does not appear as author, largely inspired the investigation and took an active part in it until his death.

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