THE INACTIVATION OF ENCEPHALITIS VIRUS (ST. LOUIS TYPE) BY MEANS OF SOFT X-RAYS

HELEN NORRIS MOORE AND H. KERSTEN

Department of Bacteriology and Department of Physics, University of Cincinnati

Received for publication December 7, 1936

Although it is well known that bacteria, yeasts, and molds can be killed by irradiation with soft x-rays, little work has been reported concerning analogous work on virus suspensions. Bruynoghe and LeFevere de Arric (1925) inactivated several viruses using the radiations from radium, while Lenz and Jungeblut (1932) reported no inactivation using hard x-rays. The lack of inactivation in this latter case may have been due to the fact that with the penetrating hard x-rays not enough of the radiant energy was absorbed by the virus or that the period of exposure was too short to absorb enough energy to cause inactivation. If it is this absorbed energy which causes inactivation, then one would expect better results with x-rays in the "soft" or long wave length region, since this type of ray is more readily absorbed. With this in mind the experiments described below were carried out using radiations of longer wave-length for longer periods of time than has been heretofore reported in the case of viruses.

METHODS AND APPARATUS

The entire irradiation technique was the same as that described by the authors (Moore and Kersten, 1936) in a previous publication. Briefly, it consists in placing the material to be irradiated in a small glass dish, 10 mm. deep and 15 mm. wide and placing the dish at a distance of 5 cm. from the focal spot of a water-cooled x-ray tube. With this technique it has been demonstrated that the temperature of the suspension being irradiated does not rise more than 3°C. above room temperature which rules out the possibility of inactivation by heat.

Mouse brain tissue, either glycerinated or fresh, was ground in a sterile mortar, transferred to the irradiation dishes, weighed and irradiated for four hours. After irradiation the brain tissue was transferred quantitatively to sterile tubes and made up to a concentration of 10 per cent based on the original weight of the sample. Either Tyrode's solution or saline was used as the diluent. The water-clear suspensions were prepared by centrifuging a 10 per cent crude suspension of brain tissue at about 2000 r.p.m. for 30 minutes and then irradiating the supernatant for 4 hours. The glycerinated tissue used was never older than 10 days and the fresh tissue was removed from the animal and used at once.

The inoculations were intracerebral (0.03 cc.) into Swiss mice weighing from 15 to 25 grams. Control animals received an equal dose of suspension prepared from brain tissue which had been kept next to the x-ray tube during the irradiation period in order to insure that temperature effects would be nearly the same.

SUMMARY

The results of 7 experiments in which 59 animals were used are summarized in tables 1 and 2. The failure of experiments 1 and 2 may be attributed to the fact that the depth of the 2 cc. of material in the irradiation dish was too great to permit the rays to penetrate with sufficient intensity to the lower layers. In these 2 experiments a total of 22 animals were used, and although they all died with typical symptoms, the average incubation period of the disease was 9 days. The control animals were 4 in number and all succumbed with typical symptoms at the end of 3 to 5 days.

In the later experiments, numbers 3, 4, 5, 6, and 7, the depth of the material was one-half that used in the first 2 experiments and the experimental animals all survived and were reinfected about 3 weeks later. They all then succumbed, indicating that there was no immunity from the previous injection and, therefore, unlikely that living virus was present in the original injection.

Experiments 6 and 7 were performed to rule out the possibility of the virus being effected by decomposition products of the brain tissue itself or the small amount of glycerin which may have adhered to the brain tissue in spite of thorough washing, or possible decomposition of the Tyrode's solution. Experiment 6 utilized glycerinated tissue suspended in saline and then centri-

TABLE 1

Showing the effects of 4 hours irradiation of encephalitis virus with soft x-rays (λ = *1.37A–1.54Å*)

EXPERIMENT NUMBER	NUMBER OF EXPERIMENTAL MICE	NUMBER OF EXPERIMENTAL ANIMALS SURVIVING	NUMBER OF CONTROL MICE	NUMBER OF CONTROL MICE SURVIVING	AMOUNT OF MATERIAL IRRADIATED	KIND OF TISSUE
					cc.	
1	12	0	2	0	About 2	Fresh
2	10	0	2	0	About 2	Glycerinated
3	6	6	2	0	1	Glycerinated
4	8	7*	4	0	1	Glycerinated
5	7	7	3	0	1	Glycerinated

* One of the 8 experimental animals died of infection. A coccus cultured from the heart's blood produced death in a susceptible animal.

TABLE 2

Showing the effect of 4 hours irradiation of purified encephalitis virus suspension with soft x-rays (λ = *1.37A–1.54Å*)

EXPERIMENT NUMBER	NUMBER OF EXPERIMENTAL MICE	NUMBER OF EXPERIMENTAL ANIMALS SURVIVING	NUMBER OF CONTROL MICE	NUMBER OF CONTROL MICE SURVIVING	AMOUNT OF MATERIAL IRRADIATED	KIND OF TISSUE
					cc.	
6	8	8	3	0	1	Glycerinated
7	8	8	3	0	1	Fresh

fuged to remove all excess tissue. The clear supernatant liquid was then irradiated. Experiment 7 used fresh tissue suspended in saline and then centrifuged to remove all excess tissue. In both cases all the experimental animals survived and all the controls succumbed, thus demonstrating that decomposition products were not a contributing factor.

CONCLUSION

Encephalitis virus (St. Louis type) has been inactivated by irradiating virus containing tissue and purified water-clear suspensions with soft x-rays for 4 hours. The inactivation has been demonstrated by inoculating susceptible animals and subsequently killing the same animals with inoculation of active virus.

The writers wish to express their appreciation to Dr. Maurice Brodie for helpful suggestions in this work.

REFERENCES

BRUYNOGHE, R., AND LEFEVERE DE ARRIC, M. 1925 Radium on neurotropic filterable viruses. Compt. Rend. soc. de Biol., **93**, 852–854.

LENZ, M., AND JUNGEBLUT, C. W. 1932 Effect of x-rays on poliomyelitis virus in vivo and in vitro. Proc. Soc. Exper. Biol. and Med., **29**, 814–816.

MOORE, H. N., AND KERSTEN, H. 1936 A preliminary note on the preparation of non-toxic Shiga dysentery vaccines by irradiation with soft x-rays. Jour. Bact., **31**, 581–584.