Effects of Acetone, Ultraviolet Irradiation and Formalin on West Nile Virus Infectivity and Immunofluoroescent Antigenicity. (28676)

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Frozen sections of infected tissues for fluorescent antibody (FA) staining are quite satisfactory for studies of pathogenesis of West Nile (WN) virus infection(1,2). Since the virus remains viable in frozen sections, there is a risk of contracting accidental laboratory infection in handling such tissue sections infected by WN, or other arboviruses (3). An effort was thus made to ascertain if infectious virus in thin sections could be inactivated without destroying either the antigenicity of the virus or the integrity of the tissue.

Materials and methods. The technique of tissue sectioning has been described elsewhere(1). A brief description follows: 8- to 10-μ sections of tissues cut in a cryostat at —20°C are placed on clean chilled microscope slides and dried at room temperature. Tissues are fixed in acetone for 10 minutes; they are used immediately or stored in 4°C refrigerator. In this study adjacent sections of the head from a suckling mouse dying of the Egypt 101 strain of WN virus infection were exposed for varying lengths of time (a) to the atmosphere (in the dark), (b) to ultraviolet light, (c) to acetone (analytical grade) at room temperature, or (d) to 10% buffered formalin at 4°C. The source of UV light was 2 GE 2G8T5 tubes situated 13½ inches above tissue and 2 tubes situated 4 inches below tissue. Exposures from above equaled about 0.20 watt per square foot, while exposures from below equaled about 1.7 watts per square foot. (However, the light from below had to travel through a wire grid and the glass microscope slide.)

At intervals (Table I) one tissue was stained with fluorescein-tagged anti-WN rabbit gamma globulin for FA studies. Another adjacent tissue was scraped off the slide and suspended in 1 ml of phosphate buffer (pH 7.0) saline. Although dried sections weighed about 0.3 mg, these suspensions were arbitrarily considered at 10^{-1} dilution. LD_{50} end points of infection were ascertained by inoculating weanling mice intracerebrally with 0.03 ml of 10-fold serial dilutions.

Results. The results are summarized in Table I. When tissues were left untreated at room temperature, infectivity was undiminished up to 24 hours but was undetectable after 4 days. The virus retained full antigenic reactivity for at least 4 days; it was still moderately antigenic after 2 weeks.

When tissues were exposed to UV light, infectivity disappeared at a rapid rate and was undetectable within 8 minutes. Viral antigenicity was not affected for 1 hour. After 6 hours of irradiation, antigenicity was barely detectable. After 2 hours, when antigenicity was only partially destroyed the color of the background of brain tissue changed from a dull greenish grey to a pronounced bluish grey.

There was a preliminary rapid drop in infectivity in tissues left immersed in acetone. Within 8 minutes 99% of the virus was inactivated. Thereafter, however, the viral infectivity appeared to have stabilized. Infectivity was still detectable even after 24 hours' immersion. Antigenicity remained undiminished for 4 days, but was barely detectable after 2 weeks' immersion.

Infectivity titrations could not be carried out on formalin treated tissue because pieces of tissues tended to slough off during formalin immersion. Additional pieces sloughed off during the subsequent removal of formalin by rinsing in water. Antigenicity of virus left in formalin remained undiminished for 2 hours but subsequently decreased and was no longer demonstrable after 24 hours.

The integrity of tissue was fairly satisfactory under all treatments. More important to tissue integrity than any fixative was the use of a good sharp microtome blade. Tissues adhered to the slide best after acetone

TABLE I. Rate of Inactivation of Infectivity and Antigenicity in 10-μ Thick Sections of West-Nile-Virus Infected Mouse Tissues.

Length of exposure	Untreated		Ultraviolet		Acetone		Formalin‡
	Inf*	FA†	Inf	FA	Inf	FA	FA
0 min	3.8	++++					
1 "			2.8	++++	2.5	++++	++++
2 "			1.2	++++			
4 "			.8	++++			
8 "			<.6	++++	1.8	++++	++++
15 "			<.6	++++			
30 "			<.6	++++	1.5	++++	++++
1 hr				++++			
2 "				+++	1.5	++++	++++
6 "				+	1.3	++++	++
1 day	3.5	++++		—	1.6	++++	¬
2 "		++++		—		++++	—
4 "	<.6	++++		—		++++	—
14 "		++				+	

* Infectivity: Neg. log of LD_{50} of 0.03 ml inoculated intracerebrally into weanling mice.

† Antigenicity: Determined by fluorescent antibody technic. ++++ = maximum intensity.

‡ pH 7-buffered formalin kept at 4°C. Results were similar whether or not the tissue was allowed to dry prior to immersion in formalin.

immersion. Untreated and UV irradiated tissues adhered almost as well. Formalin treated tissues adhered very poorly. However when undried tissues were dipped immediately into formalin the microscopic structure of cells was superior to that observed using other techniques.

Discussion. These results are in agreement with others who noted that acetone fixation preserves viral antigenicity well on frozen sections(4), while formalin fixation has a deleterious effect(5). Others, however, have reported the preservation of viral antigenicity in paraffin blocks of uncut formalin fixed tissues(6,7,8), as well as loss of antigenicity in acetone-fixed tissues(6). Whether these apparent discrepancies represent differences in viruses or differences in techniques is not clear.

The rapid destruction of viral infectivity by UV irradiation without concomitant loss of antigenicity is in agreement with other investigators(9). Immersion of WN infected tissues in acetone, limited exposure to UV light, or a combination thereof thus appear to be of considerable value in reducing infectivity while preserving viral antigenicity for FA staining.

Summary. Infectivity of West Nile virus in frozen sections was inactivated by UV light in a matter of minutes while antigenicity persisted for several hours. Immersion of infected frozen sections in acetone inactivated 99% of virus infectivity in minutes yet antigenicity was not impaired for up to 2 weeks at room temperature. In our hands fixation by formalin was found to be unsatisfactory.

1. Kundin, W. D., Liu, C., Hysell, P., Hamashige, S., *Arch. Ges. Virusforsch.,* 1962, v12, 514.

2. Kundin, W. D., *ibid.,* 1962, v12, 529.

3. Morse, L. J., Russ, S. B., Needy, C. F., Buescher, E. L., *J. Immunol.,* 1962, v88, 240.

4. Coons, A. H., *Intern. Rev. Cytol.,* 1956, v5, 1.

5. De Groot, C. J., Metzger, J. F., Smith, C. W., Hoggan, M. D., *Virology,* 1960, v12, 317.

6. Metzger, J. F., Banks, I. S., Smith, C. W., Hoggan, M. D., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 212.

7. Sabin, A. B., Messore, G., *Encephalitis,* Elsevier Publishing Co., 1961, pp. 624-626.

8. Maestrone, G., *Nature,* 1963, v197, 409.

9. Taylor, A. R., *Ann. N. Y. Acad. Sci.,* 1960, v83, 670.

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