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13608

Ultraviolet Light-Inactivated Antigens for Complement-Fixation Tests with Central Nervous System Virus Infections.

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The presence of complement-fixing antibodies has been described in the serum of individuals infected with any of a number of central nervous system viruses,[1] as well as a method for their determination that eliminates all non-specific reactions. A recent human epidemic of encephalitis was promptly diagnosed as due to Western equine en-

1 Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

cephalomyelitis virus by means of the complement-fixation reaction.[2]

The antigens prepared for the complement-fixation test as previously described were virulent. In order to diminish the risk involved in the general use of such antigens, attempts were made to render them avirulent without materially destroying their antigenicity. This result has been accomplished by exposing the virus preparations to ultraviolet light for suitable length of time, as we shall describe.

The antigens were prepared in the following manner: Batches of 4-weeks-old W-Swiss mice were injected intracerebrally with one of the viruses; when prostrate these animals were sacrificed, their brains removed, weighed, and emulsified in 0.85% saline containing 2% normal guinea pig serum heated at 56°C for 20 minutes, the proportion being 1 g of infected brain to 10 cc of diluent. This emulsion was kept in the icebox for 20 hours and then centrifuged in a horizontal centrifuge at 2500 rpm for a half hour; the supernatant was frozen and thawed 5 times in a dry ice-alcohol mixture, and finally centrifuged in an angle head centrifuge[3] at 5000 rpm for 1 hour. The supernatant, following addition of merthiolate in a dilution of 1/10,000, constituted the antigen.

Antigens thus prepared with the viruses of rabies, Eastern and Western equine encephalomyelitis, lymphocytic choriomeningitis, and St. Louis encephalitis have been tested for virulence within 1 to 3 days of preparation by intracerebral injection into Swiss mice and found virulent in dilutions of 10^{-4} for Western equine encephalomyelitis, 10^{-3} for Eastern equine encephalomyelitis, 10^{-2} for rabies, lymphocytic choriomeningitis, and St. Louis encephalitis viruses respectively. Kept in the icebox the antigens retain their virulence for long periods of time.

Formalin- or heat-inactivation of antigens thus prepared was not satisfactory; formalin made them anticomplementary and heat destroyed their antigenicity. Ultraviolet light was used as a means of inactivation with very good results, as will be shown later. The source of ultraviolet light has been a mercury vapor lamp (Hanovia); the antigens to be inactivated were placed in quartz flasks before the addition of merthiolate and the flasks shaken mechanically while being irradiated. The time required to render each different antigen avirulent was determined by inoculating mice intracerebrally with the irradiated samples.

The avirulent irradiated antigens were then tested in parallel with

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2 Casals, J., *Am. J. Public Health*, 1941, **31**, 1281.

3 Pickels, E. G., *Review of Scientific Instruments*, in press.

TABLE I.

Effect of Irradiation with Ultraviolet Light on Complement-Fixing Antigens of Some Central Nervous System Viruses.

Virus	Virulence of antigen before irradiation. Titer = 0.03 cc intracerebral dilution	Min. ultraviolet light irradiation to render antigens avirulent, min.	Anticomplementary power. Amt guinea pig serum 1:30, equivalent to 1 unit		Specificity.* Serum titer		Antigenicity. Antigen titer	
			With non-irradiated antigen, cc	With irradiated antigen, cc	With non-irradiated antigen. Homologous antigen§	With irradiated antigen. Homologous antigen§	Non-irradiated antigen	Irradiated antigen
E.E.E.	10^{-3}	90	.16	.20	1/128†	1/128	1/2‡	1/2
W.E.E.	10^{-4}	90	.10	.10	1/128	1/128	1/16	1/16
LCM	10^{-2}	40	.13	.13	1/256>	1/256>	1/2	1/2
St. Louis	10^{-2}	40	.13	.13	1/64	1/64	1/16	1/16
Rabies (fixed)	10^{-2}	40	.13	.13	1/128	1/128	1/8	1/8

*Each serum was tested against its own antigen and the 4 heterologous antigens; the first dilution of serum was 1:2 or 1:4.

†Highest dilution of serum giving a 2+ or better fixation.

‡Highest dilution of antigen giving a 2+ or better reaction. Serum used in dilution of 1:24.

§Heterologous antigen all 0.

the non-irradiated ones with regard to their properties in the complement-fixation test, namely, anticomplementary power, specificity, and antigenicity; their stability, when kept in the icebox for several weeks, is being tested.

The results of these tests are shown in Table I and can be summarized as follows:

With our special set-up, periods of time of 90 minutes are required to destroy the virulence of the Eastern and Western equine encephalomyelitis antigens, and 40 minutes with rabies fixed, lymphocytic choriomeningitis, and St. Louis encephalitis antigens.

The irradiated antigens are not anticomplementary. The titer of the complement in the presence of irradiated and of non-irradiated antigen is the same.

As shown by the highest dilution of serum exhibiting a 2 plus or better reaction, the titer of a mouse hyperimmune serum against the irradiated and the non-irradiated antigen is the same. The specificity of the reaction with the irradiated antigen is as complete as with the non-irradiated one, as shown in both instances by the absence of any crossing with the heterologous antigens.

And finally, the titer of the antigen following irradiation is the same, or only slightly lower than the titer of the non-irradiated antigen; these titers were determined by testing serial twofold dilutions of antigen with a constant amount of immune serum.

In conclusion, by exposing virus suspensions of Eastern and Western equine encephalomyelitis, rabies, lymphocytic choriomeningitis, and St. Louis encephalitis to ultraviolet light, complement-fixing antigens can be obtained that are non-infective and specific, that lack an anticomplementary effect, and have a high antigenic titer.

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Effect of 2-4 Dinitrophenol on Rat Brain Respiration at 25, and 37.5°C.*

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Measurements of the effects of graded concentrations of 2-4 dinitrophenol (DNP), at equilibrium, on oxygen consumption (concen-

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