

of thrombin formed (Fig. 3). The curves of Fig. 2 and 3 were obtained in a fibrinogen-free system, all the clotting factors being kept constant except the one to be studied (Factor VII in Fig. 2, prothrombin in Fig. 3). The thrombin formed is determined by addition of fibrinogen. The identity in the mode of action of Factor VII from plasma and serum is obvious. Fig. 3 shows that the amount of thrombin formed depends on the quantity of prothrombin in the system. As already mentioned(1) the one-stage method for quantitative determination of Factor VII (using Seitz-filtered Factor VII-free oxalated ox-plasma) yields the same results with material obtained from plasma or serum.

Chemical and physical comparison of the two proteins is not yet possible, the quantity of the products being insufficient.

Summary. Methods of purification of Fac-

tor VII from plasma and serum are outlined. The separation of Factor VII and prothrombin from plasma is accomplished by means of chromatography. The mode of action of Factor VII from plasma and serum proved to be identical.

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Response of Young Dogs to West Nile Virus. (20085)

REGINALD L. REAGAN, MILDRED ORLOWSKI, AND A. L. BRUECKNER.

From the Virus Department, Live Stock Sanitary Service Laboratory, University of Maryland, College Park.

The virus employed in this study was the B956 strain of West Nile virus furnished by the American Type Culture Collection in Washington, D. C. This virus was obtained by the above institution from Dr. K. C. Smithburn(1) of the Rockefeller Foundation Laboratories in New York. He had isolated this virus strain from a patient in Uganda, Africa and had passed it 25 times in Swiss albino mice by intracerebral inoculation. This virus has been cultivated in chick embryos(2) and in baby chicks(3). The virus, on receipt at this laboratory, was in the form of lyophilized mouse brain of the 25th passage. Three-weeks-old Swiss albino mice (Webster strain) were employed. This strain of mice was the one recommended by Webster(4) as being especially susceptible to neurotropic viruses. The dogs used were mongrels 5 to 6 weeks of age and averaged 4 to 5 lbs each.

The lyophilized mouse brain of the 25th

passage was diluted to a 10% suspension with physiological saline. Each of 6 mice was inoculated intracerebrally with 0.03 cc of this 10% suspension. The mice showed involuntary motor reactions on the 3rd day post inoculation. When these symptoms of central nervous system involvement appeared, the mice were sacrificed, and the brains were removed aseptically. The pool of infected brains was ground with alundum and diluted to a 10% suspension with physiological saline. The suspension was then subjected to 5 minutes centrifugation in a horizontal centrifuge at 2,000 r.p.m. The supernatant was used as the inoculum for initiating the virus experiment in the dogs. The virus titrated 10^{-5} in Swiss albino mice inoculated intracerebrally. The supernatant material was injected intracerebrally into 4 unvaccinated mice and into 4 mice which had been immunized previously with the 25th mouse passage

TABLE I. Response of Puppies to West Nile Virus of the 26th Intracerebral Mouse Passage, 2 Pups in each Experiment.

Route of inoculation	No. puppies showing symptoms	Incubation period (days)
Intracerebral	2	3, 4
Intranasal	1	5, 6
Intradermal	0	
Intracardiac	1	5, 6
Intraperitoneal	0	

of West Nile virus. Immunization was accomplished by intramuscular injections using high dilutions of the virus over a period of several weeks. After 3 days the unvaccinated mice showed symptoms of central nervous system involvement, while the immunized mice showed no nervous symptoms during a 21-day observation period. This confirmed the virus to be West Nile virus.

Ten healthy mongrel puppies were divided into 5 groups of 2 puppies each. Each puppy was given a 10 cc prophylactic dose of distemper antiserum in the morning and was treated with the West Nile virus in the afternoon of the same day. Puppies exposed intracerebrally or intranasally received 1 cc of the 10% suspension of West Nile virus, while the puppies exposed dermally, intraperitoneally or intracardially received 2 cc of this 10% virus suspension. After inoculation all groups were observed twice daily for the appearance of characteristic nervous symptoms. The results of this group exposure are given in Table I.

When symptoms of central nervous system involvement appeared, affected puppies were sacrificed and the brains removed aseptically. The puppy brains were ground separately with alundum and diluted to 10% suspensions with physiological saline. The suspensions

were then subjected to 5 minutes centrifugation in a horizontal centrifuge at 2,000 r.p.m. The supernatant from each suspension was injected intracerebrally into 4 unvaccinated mice and into 4 mice which previously had been immunized with the 25th mouse passage of West Nile virus. After 5 days the unvaccinated mice showed symptoms of central nervous system involvement while the immunized mice showed no nervous symptoms during a 21-day observation period. This confirmed the virus in the brain material from each puppy showing West Nile symptoms to be West Nile virus. All puppies showing no West Nile symptoms were sacrificed at the end of a 14-day observation period. Brains were removed aseptically, ground separately with alundum, and diluted to 10% suspensions with physiological saline. The suspensions were then subjected to 5 minutes centrifugation in a horizontal centrifuge at 2,000 r.p.m. The supernatant from each suspension was injected intracerebrally into 6 3-week-old Swiss albino mice. The mice showed no nervous symptoms and were discarded after a 14-day observation period.

Summary. A strain of West Nile virus isolated by Dr. K. C. Smithburn from a patient's serum in Africa and passaged intracerebrally in Swiss albino mice has been successfully transmitted intracerebrally, intranasally, and intracardially to mongrel puppies.

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