Bwamba Fever Virus and Semliki Forest Virus* in Young Dogs. (20203)

REGINALD L. REAGAN, NINALEE STRAND, AND A. L. BRUECKNER.

From the Live Stock Sanitary Service Laboratory, Maryland State Board of Agriculture, University of Maryland, College Park.

The Bwamba fever strain of virus used for this study was obtained from Dr. K. C. Smithburn of the Rockefeller Foundation Laboratories in New York. The virus was isolated from mice after the intracerebral inoculation of serum from patients. Mice develop an encephalitis after intracerebral inoculation(1).

The Semliki Forest virus (6th passage MBB26146 in serum M494744958) was also obtained from Dr. Smithburn. A viremia and encephalitis is produced in mice when inoculated with this virus by various routes. Guinea pigs, rabbits, rhesus and red tail monkeys develop the disease only after intracerebral inoculation. Chick embryos can be used to propagate Semliki Forest virus. Neutralization antibodies have been found in humans, but the virus itself has not been isolated from them(2). The experiment in young dogs was undertaken to see if they are susceptible to these 2 viruses by various routes of inoculation.

Materials and methods. Twenty Swiss albino mice (3-weeks-old) were inoculated intracerebrally with 0.03 cc of lyophilized mouse brain (Bwamba virus) of the 82nd intracerebral mouse passage. The virus received here was lyophilized mouse brain and was diluted to a 10% suspension with sterile distilled water. Twenty Swiss albino mice (3-weeks-old) were inoculated intracerebrally with 0.03 cc of lyophilized mouse brain (Semliki Forest virus) of the 6th intracerebral mouse passage using the above technic. Mice from both groups showed nervous symptoms of mild tremors and convulsions, after an incubation period of 5 to 8 days. When symptoms appeared, the mice from each group were sacrificed separately, and the brains and cords were removed aseptically. Pools of Bwamba and Semliki Forest infected mouse brains were ground with alundum, and diluted to 20% suspensions with physiological saline. The 2 suspensions were then centrifuged for 5 minutes in an angle centrifuge at 2000 r.p.m. The supernatant from each pool was used as the inoculum for initiating virus experiments in the young dogs. The virus material (Bwamba pool) was injected intracerebrally into 6 unvaccinated mice and into 6 mice which had been immunized previously with a higher dilution of Bwamba virus over a period of several weeks. After 8 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 this pool to be Bwamba fever virus. The Semliki Forest virus pool was tested in like manner (using Semliki Forest immune mice) and this pool was also confirmed to contain Semliki Forest virus. Nineteen healthy mongrel puppies of 5 to 6 weeks of age and averaging 2 to 3 lbs each were divided into 9 groups of 2 puppies each, and 1 group of 1 puppy. Two puppies of groups No. 1 to 5 were given 1 cc of a 20% suspension of Bwamba Fever virus intracerebrally, intranasally, intradermally, intraperitoneally, and intracardially. Two puppies each of groups No. 6 to 9 and 1 puppy in group No. 10 were given 1 cc of a 20% suspension of Semliki Forest virus likewise. Puppies exposed to Bwamba Fever virus were kept in an isolated room in the experimental dog kennels and puppies exposed to the Semliki Forest virus were treated in the same way. After inoculation all groups were observed twice daily for nervous symptoms characteristically found in mammals infected experimentally with these viruses.

The *results* of the Semliki Forest virus group are in Table I. When symptoms of central nervous system involvement appeared, the affected puppies were sacrificed, and their brains removed aseptically. The puppy brains were ground separately with alundum

* Approved Public Health permit to work with these viruses.

TABLE I. Response of Young Dogs to Semliki Forest Virus (Mouse-Adapted).

Route of inoculation	Exposed	Showing symptoms	Min to max* incubation period, days
Intracerebral	2	2	9–10
Intranasal	2	0	0
Intradermal	2	0	0
Intracardial	2	0	0
Intraperitoneal	1	1	7–8

* Incubation period = Time between exposure and onset of symptoms.

and diluted to a 10% suspension with physiological saline. The suspensions were then centrifuged for 5 minutes in a horizontal centrifuge at 2000 r.p.m. The supernatant from each suspension was injected intracerebrally into 6 unvaccinated mice and into 6 mice which previously had been immunized with Semliki Forest virus. After 3-4 days the unvaccinated mice showed symptoms of central nervous system involvement, while the immunized mice showed no nervous symptoms and were discarded after a 21-day observation period.

Summary. 1. A strain of Semliki Forest virus passaged intracerebrally in Swiss albino mice has been successfully transmitted intraperitoneally and intracerebrally to mongrel puppies. 2. Puppies exposed by intacerebral, intranasal, intradermal, intraperitoneal, and intracardial routes to Bwamba Fever virus and puppies exposed by intranasal, intradermal, and intracardial routes to Semliki Forest virus showed clinically no signs of nervous symptoms, and mice injected intracerebrally with brain suspensions from these puppies showed no evidence of Bwamba Fever or Semliki Forest disease.

1. Smithburn, K. C., Mahaffy, A. F., and Paul, J. H., *Am. J. Trop. Med.*, 1941, v21, 75.

2. Rivers, Thomas M., *Viral and Rickettsial Infections of Man*, 2nd Edition, 1952, 252.

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Production of Pituitary Tumors in Mice by Chronic Administration of a Thiouracil Derivative.* (20204)

GEORGE E. MOORE,† EDWIN L. BRACKNEY,‡ AND FREDERICK G. BOCK.

From the Department of Surgery, University of Minnesota Medical School, Minneapolis.

In the course of an investigation of the long term effects of the administration of large doses of I^{131} to mice, Gorbman(1) observed the development of tumors of the anterior lobe of the pituitary gland in those animals who survive complete destruction of the thyroid gland. Many of these pituitary tumors were very large with weights more than 100 times the normal pituitary weight. This observation was soon confirmed by subsequent studies from his laboratory(2) and by others (3,4). Two different opinions as to the mechanism resulting in the formation of these adenomas of the pituitary have been expressed. Goldberg and Chaikoff(5) found that U.S.P. thyroid added to the mice's diet in small amounts prevented the formation of pituitary adenomas following radiation thyroidectomy and concluded that the enlargement of the pituitary gland induced in the mouse by I^{131} injections results from hypothyroidism alone. Gorbman(2) also found that thyroxin injections reduced the incidence and size of the pituitary tumors expected to develop after I^{131} and that implants of normal thyroid gland which remained viable were effective in preventing formation of pituitary tumors. However, more recently Gorbman has postulated that it is not the hypothyroid state alone which causes the development of the tumors but that a factor related to the ionizing radiation itself may be an influence aggrevating or synergizing the thyroidectomy

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† Presently, Director of the Roswell Park Memorial Institute, Buffalo, N. Y.

‡ Clinical Fellow of American Cancer Society.