10. Ross, C. A., Proc. Soc. Exp. Biol. and Med., 1957, v96, 582.

11. Hardaway, R. M., Husni, E. A., Geever, E. F., Noyes, H. E., Burns, J. W., Ann. Surg., 1961, v154, 791.

12. Mims, C. A., Brit. J. Exp. Path., 1962, v43, 24.

13. Good, R. A., Thomas, L., J. Exp. Med., 1953, v97, 871.

14. Thomas, L., Smith, R. T., Proc. Soc. Exp. Biol. and Med., 1954, v86, 810.

15. Henle, G., Henle, W., J. Exp. Med., 1946, v84, 623.

16. Ogasawara, K., Yasne, T., Virology, 1959, v8, 268.

17. Trentin, J. J., Science, 1953, v117, 226.

Received February 25, 1965. P.S.E.B.M., 1965, v119.

The First Isolations of Powassan Virus in New York State.* (30202)

ELINOR WHITNEY AND HUGO JAMNBACK (Introduced by Rachel Brown)
Division of Laboratories and Research, New York State Department of Health, and State Museum and Science Service, New York State Education Department, Albany

Group B arbovirus activity was demonstrated in New York State by the detection of antibodies closely related to Powassan (POW) virus in wild animal and in human sera(1). The initial isolation of POW(2) virus was from a child who lived in Canada approximately 200 miles north of the New York State border. Four years later, other POW strains were recovered from *Ixodes marxi* and from the blood of a *Tamiasciurus hudsonicus* (red squirrel) collected in the same vicinity(3). The United States strains of POW virus were isolated from *Dermacentor andersoni* collected in Colorado(4) and, more recently, in South Dakota(3). The presence of Group B arbovirus in New York State has now been established by the isolation of POW virus from wild animals and ticks in northern St. Lawrence County and from a fox in Broome County, 200 miles to the south.

Materials and methods. Under the supervision of one of us (Dr. Hugo Jamnback), mosquitoes and wild animals were live-trapped in the summer of 1964 at various sites on Barnhart Island in the St. Lawrence River. Barnhart Island is separated from the mainland by 2 locks and a canal. Animals and arthropods were also collected on or near dairy farms where arbovirus activity had previously been detected(5). Ectoparasites found on the animals were removed and placed in separate tubes. Blood was collected from the animals; serum and cells were separated; sections of brain, liver, spleen, and kidney were put into separate tightly capped plastic tubes. Mosquitoes were identified and separated as to species and put in plastic tubes. All samples were stored frozen at —20°C until shipped to the laboratory.

Unless otherwise noted, methods of shipment, subsequent storage, preparation of inocula, isolation technics, identification and serologic tests were those previously described(1,6).

In the neutralization (neut.) test for antigenic comparison of strains, 2-fold serial dilutions of serum (1:4-1:256) were mixed with a constant dose of virus.

Hemagglutination (HA) antigens were prepared by the sucrose-acetone method(7). Serum inhibitors were removed by acetone treatment. Hemagglutination-inhibition (HI) tests were done by the Takatsy-loop microtechnic(8) and disposable plates† were used.

Complement-fixation (CF) antigens were HA antigens inactivated by ultraviolet light for 30 minutes. The CF method of Kent and Fife(9) which employs 5 units of complement was used. Box titrations of antigen and sera were carried out.

The baby hamster kidney cell line designated BHK21(10) was obtained from Dr.

* Supported in part by Grant 1-SO1-FR-05247 from Nat. Inst. Health.

† Cooke Engineering Co., Alexandria, Virginia.

TABLE I. Powassan Strains Isolated from Ticks and *Marmota monax* Trapped in St. Lawrence County in 1964.

Marmota monax No.	Age	Lab. No.	Specimen	Date collected	Date tested	Original isolation: Mice	Original isolation: Incubation period (days)	Titer* after one passage of virus, LD_{50}/0.03 ml
25	Not given	64-7062	2 ticks unidentified	7/ 7/64	7/15/64	7/15†	7–11	8.9
		64-7170	Blood clot		10/22/64	15/15	6– 9	8.6
		64-7158	Spleen			5/ 5	6–10	>9.0
		64-7156	Liver			14/14	6–11	8.4
		64-7157	Kidney			16/16	6–12	8.6
72	Immature	64-7562	4 *Ixodes cookei*	7/16/64	8/ 4/64	15/15	5– 6	9.2
		64-7483	Blood clot		10/22/64	16/16	6–13	8.7
		64-7479	Spleen			10/10	6–10	8.3
		64-7482	Liver			8/16	6–12	>9.0

* Titer expressed as $\log_{10}$ of number of LD_{50}/0.03 ml. † No. sick or dead/No. inoculated.

Sonja Buckley, Rockefeller Foundation Virus Laboratories. The cells were transferred weekly. The growth medium was 80% Eagle's, 10% tryptose phosphate broth, and 10% fetal bovine serum; maintenance medium was 97% Eagle's and 3% fetal bovine serum. Tris buffer was used in maintenance medium only after initial adaptation of virus and then in a concentration of 0.1 ml of 2 M Tris to 200 ml of medium.

Results. Isolation. Nine isolations of POW virus were made in 1-day-old mice from a total of 260 specimens: 113 ectoparasite pools, 78 tissues, and 69 mosquito pools (Table I). These agents came from 2 female *Marmota monax,* No. 25 and No. 72, which were trapped at 2 sites about 100 feet from the road in bushy meadowland, and approximately 2 miles distant from each other, separated by a river.

Seven of 15 mice originally inoculated with 64-7062, a suspension prepared from 2 ticks taken from *Marmota monax* No. 25, sickened between 7 and 12 days; all 15 mice inoculated with 64-7562, a suspension of 4 *Ixodes cookei* taken from *Marmota monax* No. 72, were ill after 5 to 6 days.

The incubation period was reduced to 3 days on subsequent passage of 10% suckling mouse brain suspension (smb). The agents proved infectious for 1-day-old and weanling mice by 3 inoculation routes, intracerebral (ic), intraperitoneal (ip), and subcutaneous (sc).

Guinea pigs and hamsters, however, failed to show overt signs of infection except for the group of 5 hamsters inoculated ic with 0.1 ml of 10% smb of 64-7562. Two hamsters were ill on day 7; one was sacrificed and virus was isolated from blood, liver, spleen, and brain; the second died on the 11th post-inoculation day at which time the other 3 hamsters appeared sick but recovered 2 days later.

The 2 agents readily passed through an EK Seitz pad in a Swinney filter.

The agents were sensitive to sodium desoxycholate. Untreated virus suspensions had titers of 10^9 LD_{50} per 0.03 ml; the titers after exposure were less than 10^2 LD_{50} per 0.03 ml. Ether reduced the virus titer by 3.5 $\log_{10}$.

Histologic section of the mouse brains infected with the 2 viruses showed severe encephalomalacia of the entire cerebrum, cerebellum, midbrain, and spinal cord. Margination of the chromatin was a prominent feature. No inclusions were seen.

Strains 64-7062 and 64-7562 were easily propagated in BHK21 cells. In the primary passage clumping and rounding of the cells were noted the 3rd day after inoculation at which time the tissue culture (TC) fluid had an HA titer of 40 with goose erythrocytes diluted in pH buffer 6.4 Seventeen days and 7 fluid changes later the HA titer was 320. In the third TC passage the HA titer increased to 5120 and the $TCID_{50}$ was $10^{8.5}$

TABLE II. Comparison of 2 Virus Isolates with the Original Powassan Strain.

Antigen	Complement-fixation titer			Hemagglutination-inhibition titer* 4-8 units of antigen			Neutralization titer†		
	Mouse sera			Mouse sera			Virus test dose LD_{50}	Mouse sera	
	POW	64-7062	Normal	POW	64-7062	Normal		POW	64-7062
Powassan	128/32‡	256/16	<4	1280	1280	10	124	64	32
64-7062	256/32	256/32	<4	320	320	<10	6500	8	8
64-7562	256/64	256/64	<4	640	1280	10	200	32	64
Normal suckling mouse brain	<4	<4	<4						

* Reciprocal of highest dilution of serum inhibiting hemagglutination.

† Reciprocal of highest dilution of serum protecting 75% of infected mice.

‡ $\frac{\text{Reciprocal of highest dilution of serum giving 50\% hemolysis}}{\text{Reciprocal of highest dilution of antigen giving 50\% hemolysis}}$.

per 0.1 ml which was similar to the titer in suckling mice.

Identification. Powassan immune rabbit serum protected mice inoculated with 252 LD_{50} of strain 64-7062. No neutralization was evident with undiluted antisera of the following viruses: MM, herpes simplex (HS), lymphocytic choriomeningitis (LCM), Colorado tick fever (CTF), *Aedes trivittatus,* 2 strains of Cache Valley (CV),‡ Flanders (FL), St. Louis encephalitis (SLE), Eastern and Western encephalomyelitis (EE and WE), and psittacosis.

Serologic tests by 3 methods were done to compare the original prototype POW virus (2) isolated in 1958 from a child and the 2 newly isolated tick strains. The hyperimmune mouse sera were prepared with the prototype POW virus and with strain 64-7062. Hemagglutination-inhibition, CF and neut. tests showed that the 3 agents were similar if not identical (Table II). Titers of the sera varied by no more than 2-fold with the different strains. The low titers with 64-7062 in the neut. test reflect the excessive test dose of virus.

Tissues from the 2 *Marmota monax* were tested in mice and 7 agents were isolated (Table I). These agents were identified as POW virus in neut. tests using 2 rabbit sera, one prepared with the prototype POW virus, the other with 64-7062. Both of these sera protected mice inoculated with at least 2000 LD_{50} of each strain.

‡ One is the Holden and Hess strain; the other, the Belém AR7272 strain.

No neutralizing antibodies were demonstrated for EE, WE, POW, SLE, and CV viruses in sera of animals No. 25 and No. 72 from which POW virus was isolated. In the HI test, however, the serum of No. 72 had a titer of 20 with POW antigen.

An additional strain of POW virus was isolated from the brain of a gray fox. The brain was received by the Rabies Group of the Laboratories for Veterinary Science of this Division on July 1. The animal had been found with choreiform movements in Broome County 48 hours before it had died. The brain suspension was inoculated intracerebrally into 10- to 12-g mice July 1, and an infectious agent was isolated which was submitted in its 4th passage to us for identification. In suckling mice the titer was $10^{8.5}$ LD_{50} per 0.03 ml smb suspension. Two immune rabbit sera, one prepared with POW, the other with strain 64-7062, protected all of 16 mice inoculated with 40 LD_{50} of the fox strain. No neutralization was evident with antisera of the following viruses: MM, HS, LCM, EE, WE, SLE, CTF, CV, or FL.

Summary. Powassan virus was isolated from 2 pools of ticks (1 unidentified, the other *Ixodes cookei*) removed from 2 *Marmota monax* live-trapped in northern St. Lawrence County. Powassan virus was also isolated from tissues taken from the same 2 animals. Another strain of POW virus was recovered from the brain of a sick gray fox found in Broome County 200 miles to the south in New York State. The 2 tick strains were compared antigenically with the proto-

type POW strain and were found to be antigenically similar.

Grateful acknowledgement is made to Mr. Thomas Watthews, Mr. Robert Means, and Mr. J. W. Wilcox for identification and collection of specimens; to Dr. Doris Collins for histopathologic examinations; and to Mrs. Ruth Swift, Mr. Albert Roz, and Mr. George Rayner for excellent technical assistance.

1. Whitney, E., Am. J. Trop. Med. and Hyg., 1963, v12, 417.

2. McLean, D. M., Donohue, W. L., Canad. Med. Assn. J., 1959, v80, 708.

3. McLean, D. M., de Vos, A., Quantz, E. J., Am. J. Trop. Med. Hyg., 1964, v13, 747.

4. Thomas, L. A., Kennedy, R. C., Eklund, C. M., Proc. Soc. Exp. Biol. and Med., 1960, v104, 355.

5. Whitney, E., Am. J. Vet. Res., 1965, in press.

6. ———, Am. J. Trop. Med. and Hyg., 1964, v13, 123.

7. Clarke, D. H., Casals, J., ibid., 1958, v7, 561.

8. Sever, J. L., J. Immunol., 1962, v88, 320.

9. Kent, J. F., Fife, E. H., Jr., Am. J. Trop. Med. and Hyg., 1963, v12, 103.

10. MacPherson, I. A., Stoker, M. G. P., Virology, 1962, v16, 147.

Received February 26, 1965. P.S.E.B.M., 1965, v119.

Alteration of Sex Characteristics of Turkey Poults with Diethylstilbestrol.* (30203)

CHARLES F. SIMPSON, R. H. HARMS AND H. R. WILSON
Department of Veterinary Science, University of Florida, Gainesville

Injection of male turkeys with diethylstilbestrol (DES) causes the development of aortic ruptures, hyperproteinemia, hypercholesterolemia, and hyperlipemia(1). Although lipid metabolism in the chicken appears to be controlled by the ovarian hormones, concentration of blood lipids of immature male and female birds is not remarkably different from that found in other species such as man (2). However, blood lipid levels of avian females during periods of egg formation are greatly elevated as compared to chickens not in production.

Few trials have been described in which turkeys were injected with DES for long periods of time, and there are conflicting reports concerning the value of DES in stimulating growth and fat deposition in turkeys (3). The present work was conducted to determine the influence of this estrogen on immature turkeys.

Materials and methods. Male and female Broad-Breasted Bronze turkeys were purchased at one day of age from a commercial hatchery. They were raised by routine poultry husbandry methods which have been reported previously(1). Two experiments were conducted which were identical except that they were separated by an interval of 5 months. There were 80 males and 80 females in each experiment, consisting of 16 groups, each containing 10 poults. Eight turkeys in each group were injected with DES, and 2 turkeys served as controls. Birds were started on DES at various ages. Four groups of males received the first injection of 30 mg of DES in liquid form† at 6 weeks of age, and 2 groups had an initial injection at 8 weeks. Another 2 groups were first injected at 10 weeks of age. The same numbers and groups of females were subjected to the identical treatment schedule. Treatment with DES and collection of blood serum for total cholesterol determinations(4) were repeated weekly until the experiment terminated at 18 weeks of age. Oviduct and testes of treated and control turkeys were compared.

Results. About 8 days following the first DES injection, all treated male birds began to strut, as evidenced by pronounced spreading of the tail feathers and dropping of the wing tips. Strutting was spontaneous; how-

* Supported in part by a grant from Nat. Heart Inst., U.S.P.H.S. Florida Agr. Exp. Stations Journal Series No. 2116.

† Caponade, Vineland Laboratories, Vineland, N. J.