Interrelationships Observed Between Lansing Strain of Poliomyelitis Virus and Virus of Sporadic Bovine Encephalomyelitis.* (19173)

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McNutt and Waller(1) described an acute infectious disease of cattle which is now referred to as sporadic bovine encephalitis. The disease was characterized by fever, loss of appetite, weight loss, and weakness of the hind limbs(2). As a rule, cattle less than 3 years of age were attacked. NcNutt(2) and Harshfield(3) have detected in brain tissues obtained from infected calves a filterable agent which was able to reproduce the disease. The causative agent has not been grown in artificial media, but it can be passaged in series in the yolk sac of developing chick embryos, and in the peritoneal cavity of guinea pigs. Although not completely defined, the agent appears to be a filterable virus.

Two strains of bovine encephalitis virus (BEV) have been studied in this laboratory in an effort to define a relationship between them and some other known neurotropic viruses affecting human beings. These 2 strains have been adapted successfully to Swiss mice, cotton rats, and *rhesus* (M. mulatto) monkeys Serologic tests have indicated a relationship between the virus of sporadic bovine encephalitis and the Lansing strain of poliomyelitis virus. The results of these experiments are described herein.

Materials and methods. Viruses. The "L" and "S" strains of sporadic BEV were received on November 18, 1950 from Dr. G. S. Harshfield.† Each of these strains was passaged in 7-day-old chick embryos on March 7, 1951. The yolk of moribund embryos was harvested 4 to 6 days later. Bacteria-free aliquots (2 ml) of each of the yolk pools were stored at —70°C. These materials were used in the experiments reported here. The Lansing strain of poliomyelitis virus was received from Dr. Charles Armstrong‡ on September 13, 1946, and represented the 332nd passage in mice. The virus used in the present study represented the 334th passage prepared as a pool in this laboratory on October 23, 1950.

Serums. Serums were obtained from *rhesus* monkeys before and following convalescence from infection with BEV. Immune serums were also obtained by the repeated inoculation of infected yolk emulsion and mouse brain extracts mixed with adjuvants(4). Antiserums were prepared (1948-1950) in *rhesus* monkeys with the Lansing strain of poliomyelitis virus,§ the WE strain of lymphocytic chorio-meningitis virus, Western equine encephalitis (WEE), and St. Louis encephalitis viruses. These antiserums of known titer were used also in neutralization tests. *Animal passage.* Each strain of BEV was passaged intracerebrally in Swiss mice, cotton rats, hamsters, guinea pigs, rabbits, and *rhesus* monkeys. In the event of apparent illness, or the appearance of neurological symptoms, animals were sacrificed for purposes of (a) histologic study, and (b) the passage of CNS emulsions into other animals. All animals were observed 30 days unless they were sacrificed earlier. *Neutralization tests.* In the

* Aided by a Grant from the National Foundation for Infantile Paralysis, Inc.

1. McNutt, S. H., and Waller, E. F., *The Cornell Veterinarian,* 1940, v30, 437.

2. McNutt, S. H., The *N. Am. Veterinarian,* 1942, v23, 224.

3. Harshfield, G. S., *South Dakota Farm and Home Research,* 1951, v2, 43.

† We are indebted to Dr. G. S. Harshfield, South Dakota State College, Brookings, South Dakota, for his kindness in making available to us the "L" and "S" strains of sporadic boxine encephalitis virus.

‡ We are indebted to Dr. Charles Armstrong, National Institute of Health, Bethesda, Maryland for the Lansing strain of poliomyelitis virus.

4. Salk, J. E., Lewis, J. L., Bennett, B. L., Youngner, J. S., and Bubasch, G. R., *Bact. Proc.,* 1950, p80.

§ The Lansing antiserum, A-300, was prepared by Dr. Jonas Salk at the University of Pittsburgh, Pittsburgh, Pa.

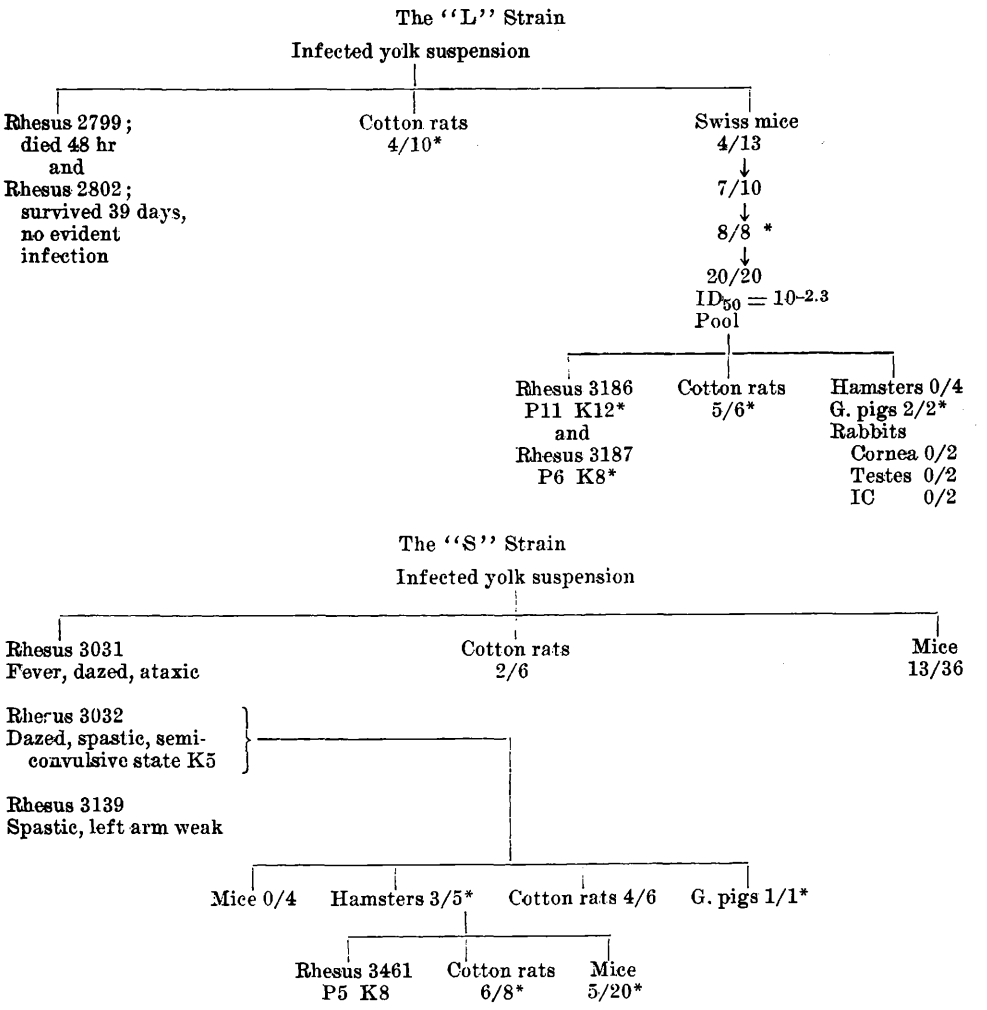

FIG. 1.

P5 = paralyzed, 5th day; K8 = killed, 8th day. * Clinical encephalomyelitis and positive histology.

serum-virus neutralization tests serial 4-fold dilutions of serum were tested against 50 or 100 ID_{50} of virus. The ID_{50} of the mouse-adapted "L" strain of BEV was $10^{-2.3}$. The ID_{50} of the Lansing strain of poliomyelitis virus was $10^{-3.9}$. Serum-virus mixtures were incubated 2 hours at 37°C. Each serum-virus mixture was inoculated into six 6-week-old Swiss mice. Appropriate controls were included. Mice were observed and scored over a period of 28 days.

Results. Adaptation of BEV to rodents and rhesus monkeys. The "L" and "S" strains of BEV caused encephalomyelitis in *rhesus* monkeys, cotton rats, and Swiss mice. Guinea pigs and hamsters experienced encephalomyelitis also after intracerebral inoculation with virus; however, infection occurred less regularly than in aforementioned animals. Domestic rabbits were not apparently affected following intracerebral or intratesticular inoculation, or by the application of virus to the scarified cornea. The "L" strain of BEV was adapted to mice, causing encephalomyelitis after cerebral inoculation. The infectivity titer was determined at the fourth passage in mice; the ID_{50} was $10^{-2.3}$. On passage in the brain of monkeys and cotton rats, the mouse-

TABLE I. Results of Neutralization Tests with BEV and Antiserums of Known Titers.

Antiserum	Homotypic serum titer	Dilution of serum 1:8	1:32	1:64	1:256	Serum protective index
WEE	1:8000	6/6*	6/6	6/6	6/6	<1:8
St. Louis	1:1000	5/6	5/6	6/6	6/6	<1:8
BEV	1:256	1/6	1/3	1/6	2/4	1:128
Lansing, A-300	>1:1024	1/6	1/6	1/6	0/6	>1:256
Normal monkey serum	<1:4	6/6	4/6	6/6	6/6	<1:8

* Numerator = No. dead or surviving; denominator = No. inoculated.
50 ID_{50} of "L" strain of BEV was used in the test.

TABLE II. The Neutralization of the Lansing Strain of Poliomyelitis Virus by BEV Immune Monkey Antiserums.

Rhesus monkey No.	Strain and antigen used for hyperimmunization	Dilution of serum 1:2	1:8	1:32	1:128	1:256	Serum protective index
2802	"L" yolk	6/6	6/6	5/6	6/6	5/6	<1:2
2810	"L" "	—*	5/6	5/6	6/6	6/6	<1:8
2944	"L" mouse brain	0/6	1/5	1/6	0/3	2/6	1:256
2947	"L" " "	0/6	0/6	1/6	3/6	3/6	1:200
3064	"S" yolk	6/6	5/6	6/6	6/6	6/6	<1:2
3065	"S" "	5/6	6/6	4/5	5/6	4/6	<1:2
A-300	Lansing, CNS	—	0/6	1/6	—	—	>1:32
Normal Pool #2	None	—	6/6	5/6	—	—	<1:8

100 ID_{50} was used.
* Mice escaped and were killed.

adapted virus produced illness and subsequent appearance of flaccid paralysis. It also provoked illness in guinea pigs, but failed to affect four hamsters. Difficulty in re-establishing the strain in chick embryos was met with. As stated earlier, rabbits were not visibly affected by the mouse-adapted strain of BEV. The "S" strain of virus was adapted also to monkeys, hamsters, mice, and cotton rats. The monkey-adapted virus caused encephalomyelitis in hamsters, cotton rats, and guinea pigs. The hamster-adapted virus in turn caused paralysis in mice, cotton rats, and a *rhesus* monkey. The "S" strain was somewhat more difficult to adapt to mice than the "L" strain, but this was accomplished with hamster-adapted BEV. A diagrammatic sketch of some of the passages made in rodents and in monkeys appears in Fig. 1.

Histologic pathology. The CNS of mice, cotton rats, and monkeys showed meningoencephalomyelitis. There was cellular damage both of neurones and supporting tissue framework In monkeys the lesions in the spinal cord were indistinguishable from those seen in poliomyelitis.

Neutralization tests. Four neutralization tests were done. The "L" strain of BEV was used in the tests. *In ovo* and mouse neutralization tests gave corresponding results. Neutralization of BEV was not observed in tests made with WEE, St. Louis encephalitis, and lymphocytic choriomeningitis antiserums. The results obtained in one of the tests appear in Table I.

It is evident from the data in Table I that BEV was neutralized by the homologous antiserum, and in addition by antiserum obtained from monkeys immunized with the Lansing strain of poliomyelitis virus. In order to study this relationship, six monkeys were immunized with BEV yolk suspensions ("L" and "S" strains) and the mouse-adapted "L" strain of BEV. These antiserums were then tested in order to determine their capacities, respectively, to neutralize the mouse-adapted Lansing strain of poliomyelitis virus. The results of the tests appear in Table II.

Monkeys immunized with mouse-adapted BEV had a high titer of serum antibody (serum dilution, 1:200 or >) able to neutralize the Lansing strain of poliomyelitis

virus. Monkeys immunized with egg-yolk suspension containing virus ($ID_{50} = 10^{-3.7}$) failed to develop neutralizing antibodies. In the latter monkeys, BEV antibodies were not found both *in ovo* and in mouse neutralization tests. The differences in the two antigens in regard to their capacities to form antibodies are being studied further.

Discussion. These observations on BEV are recorded because: (a) the virus has been adapted to new mammalian hosts, and (b) there appears to be an immunologic relationship between it and the Lansing strain of poliomyelitis virus. It should not be inferred from these data that BEV is caused by poliomyelitis virus. Further studies, now in progress, should clarify the nature of the viral agent. There is evidence(2,5) that the infectious agent of BEV may be a member of the psittacine group of viruses, since elementary bodies have been observed(2,6) in the exudates of calves sick with the disease, and in infected tissues derived from animals inoculated with BEV.

These observations are of additional interest, because they would explain heretofore perplexing observations regarding the occurrence of neutralizing antibodies for the Lansing strain of poliomyelitis virus in the body fluids of cattle(7) and other domestic birds and animals.

Summary. Two strains of bovine encephalitis virus have been adapted to mice, cotton rats, and *rhesus* monkeys causing meningoencephalomyelitis. Evidence was presented to show that the Lansing strain of poliomyelitis virus and the bovine encephalitis virus are related serologically.‖

5. Cox, H. R., and Wong, S. C., personal communication.

6. Wenner, H. A., unpublished data.

7. Hammon, W. McD., Mack, W. N., and Reeves, W. C., *J. Immunol.*, 1947, v57, 285.

‖ We have considered carefully the possibility of contamination of infected yolk suspension with the Lansing strain of poliomyelitis virus. Against this likelihood has been the fact that BEV has been passaged many times in chick embryonic tissues, that each of the strains studied caused encephalitis in hamsters and guinea pigs, and finally, that the egg-adapted strain was neutralized by Lansing serum as well as the mouse-adapted.

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Use of Trichloracetic Acid in Purification of Lipids.* (19174)

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Several methods have been developed for the removal of impurities, chiefly nitrogenous, from lipid extracts of animal tissues(1-3). That of McKibbin and Taylor(3) has been found in this laboratory to be the most satisfactory from the standpoint of general application and completeness of purification with minimal loss of lipid. The number of operations required in this method, however, reduces its desirability for use with micro quantities, and in work involving radioactive tracers. Attempts to find a suitable procedure indicated that removal of the acid-soluble material from a tissue with 10% trichloracetic acid (TCA) prior to extraction with lipid solvents may facilitate purification of the lipid extract. Swanson and Artom(4) reported the use of TCA in a similar manner, but they noted that when tissues were ex-

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1. Folch, J., and Van Slyke, D. D., PROC. SOC. EXP. BIOL. AND MED., 1939, v41, 514.

2. Sinclair, R. G., *J. Biol. Chem.*, 1948, v174, 343.

3. McKibbin, J. M., and Taylor, W. E., *J. Biol. Chem.*, 1949, v178, 17.

4. Swanson, M. A., and Artom, C., *J. Biol. Chem.*, 1950, v187, 281.