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Wallingford Poliomyelitis Virus: Another Strain of the Lansing Type, Infective in Rodents.*

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Since the original isolation by Armstrong[1] of poliomyelitis virus capable of infecting rodents, 4 other strains immunologically related to the Lansing virus and also infective in rodents have been established. One of these is the M.E.F.1. strain, isolated from the central nervous system (C.N.S.) of a fatal case in the British Middle East Forces in 1942.[2,3] Two others isolated by investigators at Yale are the S.K. and Phillips strains. The first was obtained from feces of a non-paralytic patient in New Haven in 1937[4,5] and the second was obtained from the C.N.S. of a fatal case in Egypt in 1943.[5] A fourth strain (W.W.)[6] was isolated from the blood-stream of a patient in New York in 1946, although such blood-stream isolations of poliomyelitis virus are very rare events.

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1 Armstrong, C., *Pub. Health Rep.*, 1939, **54**, 1719.

2 Van Rooyen, C. E., and Morgan, A. D., *Edinburgh Med. J.*, 1943, **50**, 705.

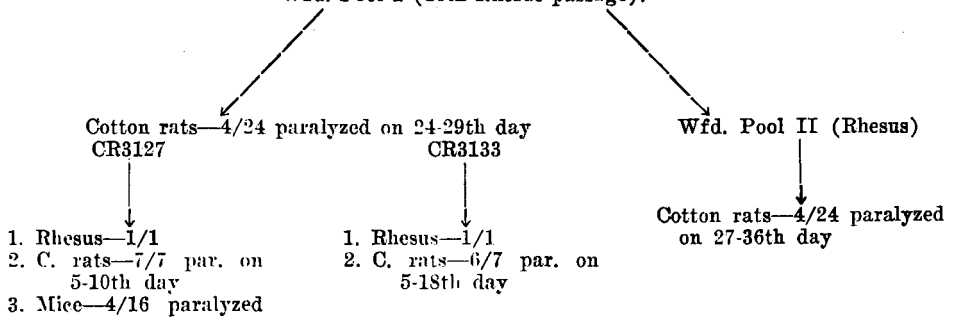
3 Schlesinger, R. W., Morgan, I. M., and Olitsky, P. K., *Science*, 1943, **98**, 452.

4 Trask, J. D., Vignec, A. J., and Paul, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 147.

5 Melnick, J. L., and Ward, R., *Fed. Proc.*, 1948, **7**, 308.

6 Koprowski, H., Norton, T. W., and McDermott, W., *Pub. Health Rep.*, 1947, **62**, 1467.

CHART 1.
Passage of 10% Wfd. Virus Suspension in Rodents.
Wfd. Pool I (16th Rhesus passage).



In the course of investigations of strain differences, in which it was clearly shown that three distinct types of poliomyelitis viruses can be identified, the suspicion arose that another strain used in this laboratory—the Wallingford (Wfd.)—might be immunologically related to Lansing virus. This strain was found to be unrelated to the Brunhilde virus, as will be shown, and since the Brunhilde strain is immunologically distinct from the Lansing, it was decided to compare the Wfd. with the Lansing strain. The Wfd. strain was isolated in California in 1934 by Trask and Paul and was soon described as a strain of unusual infectivity by intracutaneous inoculation in monkeys.[7] It was obtained in this laboratory in 1938 through the courtesy of the late Dr. James Trask, and was used in experiments to utilize its apparently high infectivity by the intracutaneous route until 1940. It was then in its sixteenth rhesus passage, having been maintained as an aqueous 20% suspension, stored in a dry-ice chest, since it was obtained in the form of glycerolated cord, in the eleventh rhesus passage, from Dr. Trask.

A pool of cords from 2 rhesus monkeys, both representing the sixteenth rhesus passage (Wfd. Pool I), and a second pool (Wfd. II) derived from 3 monkey cords infected with virus from Wfd. Pool I, were used for the rodent passage experiments. The Pool I suspension had been stored in a dry-ice chest from 1940 to 1948. Chart I summarizes the results of these rodent passages and the reverse passage back to rhesus monkeys.

It will be noted that in the first passage to cotton rats of Pool I and Pool II, not a single incubation period was less than 24 days, among 8 cotton rats paralysed. In contrast, cotton rats inoculated with material from 2 cotton rats, paralysed in the first rodent passage, had much shorter incubation periods (11 of 14 between 5 and 10 days). The cords of these 2 cotton rats each infected the single rhesus monkey inoculated therewith, and the one cotton rat cord inoculated intracerebrally in mice paralysed 4 of 16. The infected rhesus monkeys showed typical paralytic poliomyelitis and, as was the case with the cotton rats, exhibited typical poliomyelitis lesions in the brain and spinal cord.

Immunological relationship to Lansing virus. The Wfd. virus was shown to be immunologically closely related to the Lansing virus and unrelated to the Brunhilde virus (Table I). Four rhesus monkeys immunized intramuscularly with the active Brunhilde virus, and shown to be immune to intracerebral challenge with 10,000 PD 50 of the Brunhilde virus, according to the method previously described,[8] promptly succumbed to intracerebral challenge with 0.8 cc of 10^{-1} cord suspension of Wfd. Pool I. All 4 had severe paralytic poliomyelitis, confirmed by histopathological examination of the central nervous system.

Four rhesus monkeys similarly immunized with, and immune to, Lansing virus were also challenged in the same fashion with virus of Wfd. Pool I. Ten normal controls were simi-

[7] Trask, J. D., and Paul, J. R., *J. Bact.*, 1936, **31**, 527.

[8] Morgan, I. M., *Am. J. Hyg.*, in press.

TABLE I.
Immunological Relationship of Wfd. Poliomyelitis Virus to Lansing and Brunhilde Strains.

	Inoculated intracerebrally with Wfd. virus	
Immunizing virus	Convalescent rhesus monkeys	Vaccinated monkeys shown to resist immunizing virus
Brunhilde	4/4	4/4
Lansing	0/3	1/4
Normal controls	10/10	

larly challenged. All 10 controls succumbed to poliomyelitis infection, whereas only one of the 4 Lansing-immune monkeys was paralysed. Since these immunized monkeys had, because of the summer vacation, been challenged 2½ months after their last experience with Lansing virus, it was thought that a drop in antibody titer might have accounted for the failure of the one monkey to resist. Accordingly, his serum was titrated against $10^{-2.5}$ ($=10$ LD_{50}) Lansing virus in mice, and was found to have a neutralizing titer of 1 in 300. Since it is known that at this level only about 50% of monkeys are protected, and that the level of serum antibody associated with practically complete intracerebral immunity is of the order of 1 in 1,000,[9] it is likely that complete immunity of the 4 Lansing-immune monkeys to Wfd. virus would have resulted if the animals had been challenged no longer than one month after the course of immunization. A quite comparable result was obtained by challenge of monkeys with Wfd. virus several months after recovery from attacks of poliomyelitis with Lansing and Brunhilde viruses, respectively (Table I).

Discussion. It is interesting that the Wfd. virus, along with another virus (McC.) isolated in the same epidemic from nasal washings of a non-paralytic patient,[10] and the Aycock-1920 strain, were all considered to be related to the S. K. virus, as determined by neutralization tests.[11] Since the S.K. virus is immunologically related to the Lansing virus, and infective in rodents, further evidence regarding the affinities of the McC. and Aycock-1920 strains would be of interest. Aside from these 2 viruses, it appears that at least 6 other established strains are either definitely or very likely closely related to the Lansing virus by immunological test. Of these, 5 have thus far been passaged in rodents.

On the basis of intracerebral neutralization tests in mice, in which it was found that convalescent serum of monkeys infected with M.E.F.2. virus (Egypt, 1942) neutralized Lansing virus and M.E.F.2. virus, Schlesinger, Morgan and Olitsky concluded that M.E.F.2. virus was serologically of the Lansing type.[8] Our own cross-immunity experiments, however, indicate that rhesus monkeys immunized with, and immune to, Lansing virus may still be infected with M.E.F.2. virus. On the contrary, animals immunized with, and immune to, Brunhilde virus (known to be unrelated to Lansing serologically and immunologically) are completely resistant to M.E.F.2. virus.[12] This virus, therefore, cannot be considered to be primarily of the Lansing type. As shown by Schlesinger, *et al.*, it is not infective in cotton rats. Since a third strain (M.E.F.6.) studied from the Egyptian outbreak in 1942 in the Middle East Forces by the same authors, was shown by them to be serologically unrelated to the Lansing virus, it appears not unlikely from their results and from ours that 3 distinct strains produced fatal illness in that outbreak. The first, M.E.F.1., is of the Lansing type, and is infective in rodents. The second, M.E.F.2., although serologically related to the Lansing virus, at least to some extent, is more closely related to a known non-Lansing strain, the Brunhilde, by cross-immunity experi-

9 Morgan, I. M., to be published.

10 Paul, J. R., Trask, J. D., and Webster, L. T., *J. Exp. Med.*, 1935, **62**, 245.

11 Trask, J. D., Paul, J. R., and Vignec, A. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 241.

12 Bodian, D., Morgan, I. M., and Howe, H. A., *Am. J. Hyg.*, in press.

ments. The third, M.E.F.6., is apparently unrelated serologically to the Lansing strain, but more details of its affinities are needed before it can be stated that it is entirely unrelated to the Lansing.

The 9 strains either demonstrated or thought to be of the Lansing type, are listed below:

Lansing	—1938—Mich.[1]	Passaged in rodents
M.E.F.1	—1942—Egypt[3]	
Y-S.K.	—1937—New Haven[5,11]	
Phillips	—1943—Egypt[5]	
Wfd.	—1934—Los Angeles[7]	
W.W.	—1946—N. Y.[6]	
M.V.	—1909-1914—N. Y.	Not passaged in rodents
McC.	—1934—Los Angeles[10](?)	
Aycock	—1920—Vt.[11](?)	

Three of the above strains (M.E.F.1., Wfd., M.V.) have been shown in this laboratory to be not only closely related to the Lansing, but unrelated to the Brunhilde virus by tests of vaccination-immunity. Seven other strains isolated in this country from 1939 to 1946, have been shown to be related to the Brunhilde virus by the same method.[12] It seems evident, therefore, that any virus shown either to be unrelated to strains of the Brunhilde type, or related to those of the Lansing type should be investigated with respect to possible rodent pathogenicity. Such tests should be done in cotton rats rather than in mice, because of the known greater susceptibility of the former to the Lansing virus,[13] and moreover a large number of cotton rats should be inoculated. Observations of inoculated cotton rats should be continued for at least 2 months, since, as shown by Armstrong,[1] by Schlesinger, *et al.*,[3] and by our results, incubation periods may be of long duration.

It is interesting that of the six strains known to be closely related immunologically to the Lansing strain, five have been passaged in rodents. This fact leads to the strong suspicion that rodent pathogenicity is closely linked to the specific antigenic structure of this group of viruses. At first sight the MV strain appears to be an exception, but since it has had a long series of monkey passages since its isolation some 40 years ago, it may be that it originally possessed the property of rodent infectivity, but lost it because of numerous monkey transfers. Another question which may be raised is whether strains which are known not to be related immunologically to the Brunhilde virus are likely to be related to the Lansing strain. It was this supposition which led to the successful passage of Wallingford virus in rodents. Following the same suggestion, an attempt was made to passage the Leon strain in cotton rats because of our finding that it was not related to the Brunhilde virus, by tests of vaccination-immunity. This strain was isolated in Los Angeles in 1937, by Dr. John F. Kessel, who kindly supplied our sample. We have failed to passage the Leon strain in cotton rats, and moreover have shown that it is immunologically unrelated to both the Brunhilde and Lansing viruses.[12] The Leon strain is therefore representative of a third distinct immunological group of poliomyelitis viruses. It will be of interest to know whether other representatives of this group will be identified and whether they also lack the property of rodent infectivity.

It is of interest that when known Lansing-like viruses are examined as a group, they appear to be widely distributed geographically, and can be isolated from both paralytic and non-paralytic cases. Since they further occur in nasopharynx and feces, as well as in the C.N.S., and since Lansing antibodies are widely distributed in human serum, there seems to be no reason to doubt at the present time, that these viruses are important in the epidemiology of poliomyelitis. It remains to be seen whether they are of less importance in this regard than viruses of the non-Lansing type.

Summary. The Wallingford virus, isolated from the nervous system of a fatal poliomyelitis case in California in 1934, has been successfully passaged in cotton rats and in mice. Material from each of 2 paralysed cotton rats produced typical poliomyelitis in one rhesus monkey. The virus has been tested for immunological relationships by inoculation in monkeys vaccinated with, and shown to be immune to, the Lansing and the Brunhilde viruses, respectively. These two viruses are

[13] Bodian, D., Morgan, I. M., and Schwerdt, C. E., to be published.

representatives of 2 distinct types of poliomyelitis virus. The Wallingford virus is indistinguishable from the Lansing virus by the vaccination-immunity test, and unrelated to the Brunhilde virus by the same test.

A relationship between the property of rodent infectivity and the immunological specificity of the Lansing group of poliomyelitis viruses is suggested, in view of evidence for the existence of three distinct immunological groups of poliomyelitis viruses, only one of which has thus far been shown to be infective in rodents.

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Transmission of the Hamster-Adapted Newcastle Virus to Swiss Albino Mice.

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Following the adaptation of Newcastle virus, California Strain No. 11914, to the Syrian hamster,[1] attempts to transmit the modified virus of the 8th hamster passage to Swiss albino mice were successful through the fourth serial subinoculation only.[2] Brandly, *et al.*[3] working with egg propagated Newcastle virus reported similar results.

Further attempts to establish the infection in Swiss albino mice through the injection of hamster-adapted Newcastle virus of later hamster passages were likewise unsuccessful beyond several subinoculations.

Concentrated virus of the 203rd hamster passage was prepared from 8 infected hamster brains. The brain tissue was ground with alundum and diluted to a 10% suspension with physiological saline solution. Eighteen cc of the suspension were centrifugated under aseptic conditions at 50,000 RPM for 2 hours in a Spinco ultra-centrifuge. The supernate was discarded and the sediment was resuspended in 3 cc of physiological saline solution. Approximately .05 cc of the concentrated virus suspension was injected intracerebrally into each of 12 Swiss albino mice CFW.*

The use of this concentrated virus of the 203rd passage for the initial mouse inoculation established an infection in which slightly more than 10% of the injected mice of the first 8 passages showed symptoms of central nervous system involvement. From the 9th through the 20th passages more than 60% of the injected mice showed the characteristic symptoms. Table I shows the results of these 20 serial passages.

In mice responding to the virus injection irritability and malaise appeared in 2 to 9 days. In most cases these symptoms were followed by paralysis, evidenced first in the forelegs and followed in some cases by complete prostration within several hours. In other cases complete paralysis did not appear for as long as 24 hours after the forelegs became affected. Rhythmical jerking of groups of muscles or the entire body of paralyzed mice was frequently noted, accompanied by labored respirations. Death occurred within a few hours after the animals became moribund.

Brain material for passage was chosen from mice sacrificed when nervous symptoms developed. The virus-bearing brain material was ground with alundum and diluted to 10% suspension with physiological saline solution.

[1] Reagan, R. L., Lillie, M. G., Poelma, L. J., and Brueckner, A. L., *Am. J. Vet. Res.*, 1947, **8**, 136.

[2] Reagan, R. L., Lillie, M. G., Poelma, L. J., and Brueckner, A. L., *Am. J. Vet. Res.*, 1947, **8**, 427.

[3] Brandly, C. A., Moses, H. E., Jungherr, E. L., and Jones, E. E., *Am. J. Vet. Res.*, 1946, **7**, 289.

* The Webster strain of Swiss albino mice from Carworth Farms, Rockland Co., N. Y., weighing between 10 and 12 g were used for all passages.