have recently been utilized in an attempt to relate species differences in permeability to possible chemical differences in the cell membrane (Dziemian[2] and Ballentine.[3])

Because of their possible use in future studies, and for comparative purposes, the data in Table I are presented giving temperature coefficients of hemolysis of nucleated erythrocytes of several species.

The technic employed in these experiments was essentially that used by Jacobs *et al.*[1] In general, the temperature coefficients of hemolysis for these nucleated erythrocytes are of the same order of magnitude as those obtained using mammalian red cells. These data are too fragmentary to justify a detailed analysis. However, certain comparisons can well be made with data described by Jacobs and Glassman.[4] These authors point out that in fishes (elasmobranchs and teleosts) the permeability to ethylene glycol is high, in birds the permeability to ethylene glycol and glycerol is very great and nearly equal, and much less to urea, except in the duck and chicken, and permeability to urea is great in reptiles. The present data are in complete agreement. Ethylene glycol permeability is high in the four fishes studied, urea and glycerol permeability is high in the chicken, and the turtle exhibits a very high permeability to urea.

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Isolation of St. Louis Encephalitis Virus from a Fatal Human Case in California.*†

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Hammon and Reeves[1] have recently reported recovery of the St. Louis encephalitis virus from naturally infected mosquitoes (*Aedes dorsalis* Meigen) caught in Kern County, California, in 1944, the first isolation of this virus from any source in this state.

The present report describes the isolation of the St. Louis virus from a fatal human case in California. This is the first time the virus has been isolated from any vertebrate host outside the endemic St. Louis area, and furnishes additional, more direct, evidence to that obtained from serologic studies,[2-4] of the activity of this virus in California.

The patient was an itinerant agricultural worker who, during the past year, had resided in Kern County in the neighborhood

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† The author is indebted to Dr. John J. Sippy, District Health Officer of the San Joaquin Local Health District, for clinical and epidemiologic data, and to Dr. Elmer W. Smith, Pathologist to the San Joaquin General Hospital, for information on the clinical and post-mortem findings and for furnishing pathologic material for examination.

[1] Hammon, W. McD., and Reeves, W. C., *Am. J. Pub. Health*, 1945, **35**, 994.

[2] Wynns, H. L., and Hawley, C. J., *Am. J. Pub. Health*, 1939, **29**, 781.

[3] Howitt, B. F., *Am. J. Pub. Health*, 1939, **29**, 1083; ibid., 1942, **32**, 503.

[4] Hammon, W. McD., Reeves, W. C., and Galindo, P., *Am. J. Hygiene*, 1945, **42**, 299.

of Bakersfield and Arvin. He arrived in Stockton (San Joaquin County) on September 4 or 5, 1945, and lived at an auto trailer camp about 6 miles east of the city. On the morning of September 11 he was observed to be irrational and to walk with a stumbling gait, and was hospitalized with a diagnosis of acute encephalitis of undetermined etiology. Examination of the spinal fluid on admission showed the following: 10 red cells, 20 white cells, Pandy's reaction +, Gram stain for organisms negative, sugar 81.4 mg per 100 cc, chloride 720 mg per 100 cc, Kolmer's test 4 +, colloidal gold curve 0-0-1-2-1-0-0-0-0. Blood examination on admission showed: hemoglobin 83 percent, white blood cells 20,900, polymorphonuclears 76, lymphocytes 14, mononuclears 10, stabs 5, Kolmer and Kline tests both 4 +. The temperature at the time of admission was 107.4°; under treatment with medication and icecaps it dropped to 103° by the 3rd day, then rose to 105.3° at the time of death on the 4th hospital day. On post-mortem examination there was found some passive congestion of the lungs, a few scars in the liver, and an acute congestion and severe edema of the brain; the rest of the organs showed nothing unusual.

A 20 percent suspension of brain tissue in 10 percent normal rabbit serum broth was prepared and spun at 1500 r.p.m. for 15 minutes in an angle centrifuge. The supernatant fluid was inoculated intracerebrally (0.1 ml) into 2 guinea pigs, intraperitoneally (0.03 ml) into 8 mice 14 days of age, and by the combined intracerebral (0.03 ml) and intraperitoneal (0.1 ml) routes into 8 mice 20 days of age. Neither guinea pig showed evidence of infection during the one month observation period. Of the 14-day-old mice, one was found dead on the 8th day and one on the 9th day; 2 were ill on the 9th day and were found dead on the 10th day; and 4 survived and were discarded on the 21st day. Of the 20-day-old mice, one was found dead on the 5th day and the remaining 7 were obviously ill on the 7th day, showing ruffled fur, hunching, tremors, and ataxia. Four of these animals were killed, and their brains were pooled and passaged by combined intracerebral-intraperitoneal inoculation into 14- to 15-day-old mice; the remaining 3 animals were killed on the 12th day and their brains similarly inoculated in 15-day-old mice. In both cases the passage mice showed ruffling of the fur, convulsions, tremors, ataxia, and paralyses of hind or fore limbs after an incubation period of 4 to 6 days.

Aerobic and anaerobic bacteriologic cultures of infected brains were either sterile or occasionally showed the presence of a few saprophytic bacteria. Since Berkefeld N and W, and Seitz EK filtrates were bacteriologically sterile yet highly pathogenic for mice, the agent was regarded as a virus. For convenience, it has been designated the Winkler strain.

In an attempt to identify the agent, guinea pigs and hamsters were inoculated intracerebrally (0.1 ml), intraperitoneally (0.5 ml), or by both routes combined, with a 1 percent suspension of brains from the 3rd mouse passage. Three animals were inoculated by each route; all survived without showing any evidence of infection during a 3-week observation period. The lack of pathogenicity for guinea pigs was considered possibly to rule out several viruses, and the failure to produce apparent infection in hamsters to rule out others, including the St. Louis virus, to which this species has been reported as susceptible.[5,6] Nevertheless, on the assumption that newly isolated strains of a virus might fail to produce obvious infection in species normally susceptible to highly passaged laboratory strains of the same virus, neutralization tests were done using the Winkler virus and rabbit or guinea pig immune sera to the Eastern equine encephalomyelitis, Western equine encephalomyelitis, lymphocytic choriomeningitis, Japanese B, St. Louis (Hubbard strain) and Winkler viruses, and to an unidentified virus isolated from mosquitoes of California by Hammon

[5] Broun, G. O., Muether, R. O., Mezera, R. A., and LeGier, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 601.

[6] Lennette, E. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 178.

TABLE I.
Effect of Age of Animal and Route of Inoculation on the Isolation of St. Louis Encephalitis Virus in Mice.

Route of Inoculation	Age of mice: 14 days Mortality ratio*	14 days % Mort.	14 days A.S.T.†	21-28 days Mort. ratio	21-28 days % Mort.	21-28 days A.S.T.	6-7 weeks Mort. ratio	6-7 weeks % Mort.	6-7 weeks A.S.T.
			days			days			days
I.P.	20/24	83	11.0	5/27	18	18.9	3/18	17	19.0
I.C.	16/18	88	9.8	16/26	62	14.9	8/21	38	17.1
I.P. + I.C.	13/14	93	11.1	14/21	67	15.9	11/23	48	16.7

* Mortality ratio: the numerator represents the number of mice that died, the denominator the number of mice inoculated.

† A.S.T.: average survival time, computed on the basis of a 21-day observation period, according to the method devised by Kerr and described by Bugher.[9]

and Reeves.[7] The tests were set up according to the technic previously described.[8] Undiluted sera were mixed with dilutions of virus and inoculated intraperitoneally in 0.03 ml amounts into mice 14 days of age; a group of 4 mice was used for each serum-virus mixture. The immune serum against the Hubbard strain of St. Louis virus neutralized 300,000 LD_{50} of the Winkler virus. The homologous Winkler immune serum neutralized >300,000 LD_{50} and the remaining sera neutralized 10 LD_{50} or less of the virus.

Neutralization tests were then done in the reverse direction, using the Hubbard strain of St. Louis virus and Winkler and Hubbard strain immune sera. Both sera neutralized >100,000 LD_{50} of the known St. Louis virus.

The Winkler virus was therefore regarded as a strain of St. Louis encephalitis virus. Inasmuch as the patient became ill within 6 to 7 days after his arrival in San Joaquin County, it is not impossible that he may have acquired his infection in Kern County. This is a point of some interest since, as mentioned above, Hammon and Reeves[1] in 1944 had found mosquitoes in Kern County to be infected with St. Louis encephalitis virus.

The Winkler virus was subsequently isolated in 2 additional instances in which the pathogenicity of the original human material was tested in mice of different ages. Mice of 14 days of age, and of 21 to 28 days and 6 to 7 weeks of age were inoculated intracerebrally (0.03 ml), intraperitoneally (0.1 ml), or by both routes, with a 10 percent suspension of human brain tissue; the animals were examined daily during a 4-week observation period to record the number of animals sick or dead.

The results of both experiments are summarized in Table I. As is indicated by the mortality data, the 14-day-old mice were much more highly susceptible to infection by the intraperitoneal route than were the older mice. Similarly, the 14-day-old animals were more susceptible to infection by the intracerebral or combined intracerebral-intraperitoneal routes than were the older animals; the difference in the mortality between the 2-week-old and the 6- to 7-week-old animals is especially striking, and quite different from that observed with a highly passaged laboratory strain of St. Louis virus inoculated intracerebrally.[10] The average survival times[9] also point to the decreasing susceptibility with age to infection by the several routes used. It appears, therefore, that for the isolation of certain viruses, at least, the use of young animals is desirable, and that if older animals are employed somewhat larger numbers should be inoculated to compensate for the lower degree of susceptibility. It would seem, also, that combined inoculation by 2 routes is a desirable procedure to enhance

[7] Hammon, W. McD., personal communication. The antiserum to this virus was kindly supplied by Dr. Hammon.

[8] Lennette, E. H., and Koprowski, H., *J. Immunol.*, 1944, **49**, 375.

[9] Bugher, J. C., *Am. J. Trop. Med.*, 1940, **20**, 809.

[10] Lennette, E. H., and Koprowski, H., *J. Immunol.*, 1944, **49**, 175.

TABLE II.
Influence of Age on Susceptibility of Hamsters to Infection with the Winkler Strain.

Inoculum*	Mortality ratios Age of test hamsters 2-3 days	4 days	6-7 days	10-13 days	15-16 days	6-8 weeks†	6+ mo‡
10% human brain	0/5	0/5	0/4			0/3	
10% 3rd passage mouse brain	9/9		10/10	13/13	16/29	1/12	1/10

* Inoculation was done by the intracerebral route; animals up to 16 days of age were given 0.03 ml, older animals 0.1 ml.
† Average weight of animals in this group was 53.8 g.
‡ " " " " " " " " 154 g.

the possibility of infection.

As mentioned above, hamsters inoculated with a suspension of the human brain survived without obvious signs of infection. Since hamsters are highly susceptible[5,6] to infection with laboratory passage strains of the St. Louis virus, the apparent lack of pathogenicity of the Winkler strain for this species was investigated. Hamsters of various ages were inoculated intracerebrally with 10 percent suspensions of the original human brain material or of 3rd mouse passage brains; animals up to 16 days of age received 0.03 ml, the older animals 0.1 ml. The results are brought together in Table II.

As is shown in Table II, hamsters as young as 2 to 3 days of age resisted infection with the original human brain material. After 3 passages in mice, however, the virus proved to be highly lethal for young hamsters but not for the older animals. Table II shows that 3rd mouse passage virus was uniformly lethal for hamsters up to 10 to 13 days of age and that the break in susceptibility occurred at about 15 days of age, since only 16 of 29 animals of 15 to 16 days of age succumbed to the infection. Animals 6 to 8 weeks of age or older were practically insusceptible; only 2 of 22 animals in this group died, one with and one without obvious evidence of illness on the day preceding death.

On the possibility that the local hamster strain might represent one resistant to the St. Louis virus, the Hubbard strain of virus, used in previous studies[6] on the susceptibility of hamsters to St. Louis virus, was titrated in animals 6 to 8 weeks of age. Serial tenfold dilutions of a 10 percent suspension of 100th mouse passage virus were prepared in serum broth and inoculated intracerebrally in 0.1 ml amounts; 2 animals were used for each dilution. The Hubbard strain proved to be uniformly lethal for local hamsters through a dilution of 10^{-7}, the highest tested. The resistance of hamsters older than 15 days to lethal infection with the Winkler virus was therefore considered to be due to the low pathogenicity, presumably associated with the low number of passages, of this strain, and not to a refractory state peculiar to the strain of hamsters used.

Hammon[11] has observed that freshly isolated strains of Western equine encephalomyelitis virus may show a complete lack of pathogenicity for guinea pigs, similar to that described here for the Winkler St. Louis virus in hamsters. Much of the recorded information on the susceptibility of various animal species to a given virus is based on the use of highly passaged laboratory strains. Failure of original source material to induce apparent infection in an animal species used for primary isolation of a virus, and generally regarded as susceptible to that virus, or of freshly-isolated, low-passage virus to produce overt infections in such species, should therefore be accepted with reservations and not used as an infallible criterion for the elimination or inclusion of certain viruses attendant on attempts at identification.

Summary. The virus of St. Louis encephalitis was isolated from a fatal human case of encephalitis in California. This represents the first isolation of this virus from any vertebrate host outside the endemic St. Louis area, and the second isolation of this virus in California.

[11] Hammon, W. McD., personal communication.

It is considered probable that the patient acquired his infection in Kern County, where Hammon and Reeves have shown the existence of naturally infected mosquitoes.

The pathogenic properties of the virus are described and discussed.

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Elimination in Human Feces of Infectious Hepatitis Virus Parenterally Introduced.*

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Experiments in the transmission of *infectious hepatitis* and *homologous serum jaundice* to human volunteers have revealed certain similarities and differences in the properties and distribution of virus, and route of infection in these 2 conditions. These points have been summarized recently by Neefe *et al.*[1] The etiologic agents of both conditions are filtrable, resistant to a temperature of 56°C for 30 minutes, and transmissible to human volunteers in serial passage.[2-5]

In contrast to these similarities are certain differences: namely; (1) the etiologic agent of infectious hepatitis is present in the serum and feces of patients in the acute phase of the naturally occurring or experimentally produced (by feeding) disease.[6-11] On the other hand attempts to demonstrate the etiologic agent of homologous serum jaundice in the *stools* of patients with this disease have been unsuccessful.[1,11,12] (2) Infectious hepatitis may be produced in human volunteers by *feeding* or parenteral inoculation of infectious material.[2,6-11,13] In contrast, while homologous serum jaundice has been produced experimentally by parenteral inoculation of infectious serum,[3-5,9,14] attempts to transmit this condition by feeding similar material have, with one exception, been unsuccessful.[1,5,12] This one exception is an experiment of MacCallum and Bauer[4] who produced the disease in a human volunteer by feeding infectious serum.

In view of these apparent differences it appeared possible that the *route of inoculation* might affect the distribution of virus and thereby constitute an artificial difference between these 2 conditions in experimental subjects. Failure to recover virus from the stool of patients with homologous serum jaun-

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