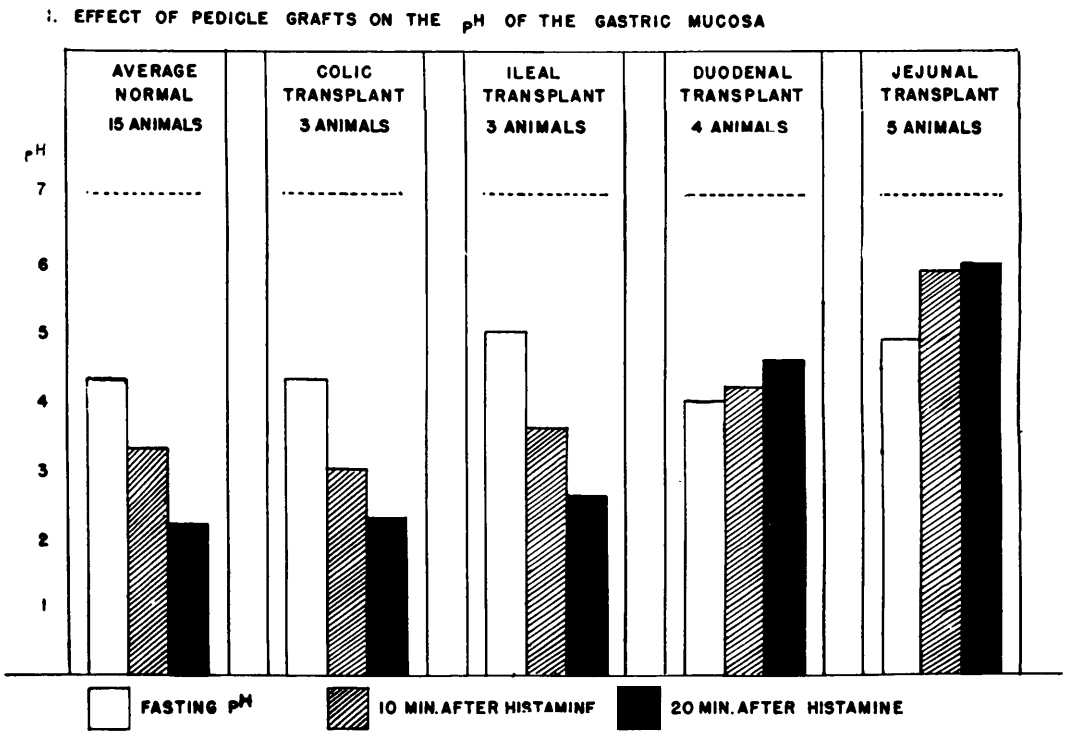

FIG. 1.

14044

Susceptibility of Hamsters to Peripheral Inoculation of Western, Eastern, and West Nile Encephalitis Viruses.

DENNIS W. WATSON AND JOSEPH E. SMADEL.
From the Hospital of The Rockefeller Institute for Medical Research.

Recent developments in the field of neurotropic virus diseases have changed a number of the prevalent ideas regarding the mode of transmission of these infections.[1] Arthropods have now been collected in the wild state infected with the viruses of Russian,[2] Japanese,[3] Western,[4,5] and St. Louis encephalitis.[5] Furthermore, laboratory studies have shown that Ixodes ticks infected with the Russian agent,[2,3b] and *Culex tarsalis* mosquitoes infected with St. Louis encephalitis[6] can transmit these diseases by biting. In addition, Japanese encephalitis may be transmitted by the bite of infected *Culex* mosquitoes[7] but two

1 Smadel, J. E., *U. S. Naval Med. Bull.*, 1942, **40**, 1020.

2 Shubladse, A. K., and Serdiukova, G. V., *Arch. Sci. Biol.*, 1939, **56**, 121.

3 a. Petrischeva, P. A., and Shubladse, A. K., *Arch. Sci. Biol.*, 1940, **59**, 72; b. Smorodintzev, A., Neustrojev, W., and Chagin, K., *Zhurnal. Mikrobiol. epidemiol. i immunobiol.*, 1941, No. 2, 3.

4 Kitselman, C. H., and Grundmann, A. W., *Kan. State College of Agriculture, Experimental Station, Tech. Bull.*, 1940, No. 50, 5.

5 Hammon, W. McD., Reeves, W. C., Brookman, B., and Izumi, E. M., *J. Infect. Dis.*, 1942, **70**, 263.

6 Hammon, W. McD., and Reeves, W. C., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 142.

7 Mitamura, T., Yamada, S., Hazato, H., Mori, K., Hosoi, T., Kitaoka, M., Watanabe, S., Okubo, K., and Tenjin, S., *Trans. Soc. Path. Jap.*, 1937, **27**, 573.

TABLE I.
Death of Hamsters Infected by Peripheral Routes.

Virus	Route of inoculation	Age of hamsters	MLD of suspension					
			1	10	100	1,000	10,000	100,000
Western	Subcut.	Old adult			0/2	2/3	2/3	1/2
	Intraperit.	" "			3/3	2/3	2/3	2/3
	"	4-5 weeks		4/4	4/4	4/4		
	"	" "		2/4	3/4	4/4		
Eastern	Subcut.	Young adult	1/2	2/2	1/2	2/2	2/2	
West Nile	"	" "	1/2	2/2	2/2	1/2	2/2	
	Intraperit.	" "	2/4	3/4	4/4			

MLD estimated by intracerebral mouse titration.
Numerators indicate number of animals dead while denominators indicate number inoculated.

groups of workers[3a,8] have failed to confirm this observation. Transmission in the laboratory of equine encephalitis virus by infected *Aëdes aegypti* was accomplished a number of years ago.[9a]

A number of the neurotropic viruses can induce disease in susceptible animals when inoculated by routes other than intracerebral or intranasal;[1,9,10] however, in relatively few situations does death of the host regularly occur following the introduction of minute amounts of these agents. The notable exceptions are guinea pigs injected by any peripheral route with the virus of choriomeningitis,[11] and baby mice inoculated intraperitoneally with the viruses of Eastern and Western encephalitis.[12] Recently Syrian hamsters have been found susceptible to the West Nile agent and to the viruses of Eastern and Western encephalitis;[13] the present report deals with the fatal disease induced in this species by the peripheral inoculation of these 3 agents.

Strains of the viruses of Eastern, Western and West Nile encephalitis employed in the present work were the same as those used earlier.[13] The agents had been through 8, 5, and 4 serial intracerebral passages, respectively, in hamsters. Suspensions of brain tissue from sick hamsters were stored at −70°C while their infectivity was determined by intracerebral titration in adult Swiss mice. Suspensions for inoculation into hamsters were then prepared so that each 0.025 cc contained a given number of minimal lethal doses (MLD) calculated on the basis of the mouse titration. Hamsters, preferably adolescents of uniform size and about 4-5 weeks of age, were generally injected intraperitoneally with 0.2 cc of inoculum but sometimes the subcutaneous route was used in titration experiments. The distribution of the three viruses in the blood and organs of hamsters at various times after inoculation was also studied. For this purpose groups of 6 or 8 hamsters were infected with one of the viruses and individual animals were sacrificed on each successive day. The virus content of heparinized plasma and of suspensions of spleen and brain tissue from these animals was determined by intracerebral titrations in mice. Histological sections were prepared from tissues of 20 hamsters which were sacrificed when moribund or autopsied within a few hours after death.

Large amounts of the Eastern or Western viruses inoculated intracerebrally produced death of hamsters in 24 to 36 hours.[13] Following peripheral inoculation of these agents the animals appeared healthy for 4-8 days, depending on the amount of virus given; how-

8 Webster, L. T., Clow, A. D., and Bauer, J. H., *J. Exp. Med.*, 1935, **61**, 479.

9 a. Kelser, R. A., *J. Am. Vet. Med. Assn.*, 1933, **82**, 767; b. Howitt, B., *J. Infect. Dis.*, 1941, **67**, 177; c. Hammon, W. McD., *J. A. M. A.*, 1942, **118**, 66; d. Merrill, M. H., and Ten Broeck, C., *J. Exp. Med.*, 1935, **62**, 687; e. Ten Broeck, C., *Arch Path.*, 1938, **25**, 750.

10 Smithburn, K. C., Hughes, T. P., Burke, A. W., and Paul, J. H., *Am. J. Trop. Med.*, 1940, **20**, 471.

11 Rivers, T. M., and Scott, T. F. McN., *J. Exp. Med.*, 1936, **63**, 415.

12 Sabin, A. B., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 597.

13 Havens, W. P., Watson, D. W., Green, R. H., Lavin, G. I., and Smadel, J. E., *J. Exp. Med.*, 1943, **77**, 139.

TABLE II.
Distribution of Virus in the Syrian Hamster.

Virus	Inoculated	Day	MLD of virus*		
			Blood	Spleen	Brain
WEE	Intraperitoneal	1	0	0	0
	10^2 MLD	2	10	10	0
		3	0	0	0
		4	0	0	$>10^4$
EEE	Intraperitoneal	1	> 1	0	0
	10^4 MLD	2	0	0	0
		3	10^2	10^2	$>10^4$
WN	Intraperitoneal	1	0	0	0
	10^3 MLD	2	10	0	0
		3	0	0	0
		4	0	10	0
		8			$>10^4$
WN	Subcutaneous, 10 MLD	8		0	$>10^4$
	10^4 MLD	10	0		$>10^4$

*MLD represents minimal lethal doses on intracerebral inoculation in mice.
0 indicates that no virus was demonstrable in a 10% suspension of tissue or in undiluted heparinized plasma.
> = greater than.

ever, once signs of illness had developed death generally occurred in a matter of hours (Table I). Clinical evidence of disease was usually limited to weakness, tremors, and roughened fur; salivation and conjunctivitis infrequently occurred and rarely, partial paralysis of one or more extremities was noted. Although neuro-pathological lesions varied in intensity they were generally less severe than those observed in hamsters inoculated intracerebrally with these agents. Sometimes only a mild lymphocytic reaction in the meninges or choroid was found but more often a few narrow cuffs of lymphocytes were observed about vessels in the brain. Occasionally, heavy infiltrations of lymphocytes were seen in Virchow-Robin spaces, and glial proliferation occurred in adjacent brain tissue where necrobiosis of nerve cells and neuronophagia were also encountered. No significant lesions were observed in histological sections of cord from three members of the group. Hemorrhagic pneumonia typical of that associated with virus infections[14] occurred in 5 of the hamsters; cultures of the lung tissue on ordinary media were bacteriologically sterile.

The disease caused by the West Nile virus developed relatively slowly whether intracerebral[13] or peripheral routes of inoculation were employed. Nevertheless, death of most animals occurred 7 to 10 days following the introduction of even small amounts of virus by the latter route (Table I). This infection was characterized in the hamster not only by a longer incubation period than that of the equine encephalitides, but also by a more striking clinical picture. Some hours preceding death the animals developed a spastic gait and hunched their backs into a kyphotic position, exhibited coarse tremors of the extremities, and readily developed convulsions on stimulation. When an occasional animal survived for several days after the onset of obvious disease, paralysis of the hind legs usually developed. Histological changes in the brains of 7 hamsters infected with this virus were somewhat more severe than the lesions encountered in the animals inoculated with the equine agents but were of essentially the same type. A few perivascular cuffs and a moderate meningeal reaction occurred in the cords of 2 of the animals.

Preliminary results indicated that old adult hamsters were less susceptible to the viruses than young adult or adolescent animals; furthermore, that variations in the response of individual animals were greater in the older age group. The most consistent titration

[14] McCordock, H. A., and Muckenfuss, R. S., *Am. J. Path.*, 1933, **9**, 221.

results were obtained when young hamsters of uniform size and an age of about 4 to 6 weeks were used.

All 3 of the agents under investigation are known to appear in the circulating blood of certain vertebrates during the course of infection; the equine agents are usually present for only a brief period early in the disease,[9] while the West Nile agent remains for a longer time.[10] The results of experiments designed to determine the distribution of virus in the blood and tissues of hamsters infected with each of the 3 agents are summarized in Table II. It is apparent from the data presented that these 3 viruses appear in the blood of hamsters within 24 or 48 hours after intraperitoneal inoculation; furthermore, that virus is demonstrable in the blood or spleen before it is detectable in appreciable quantities in the brain; and finally, that the late development of large amounts of virus in the brain is associated with a diminution or absence of virus in the blood and spleen.

Conclusions. The Syrian hamster readily becomes infected when small amounts of the viruses of Eastern, Western, or West Nile encephalitis are inoculated by peripheral routes. The viruses appear in the circulating blood of the infected animals and the diseases usually terminate fatally.

The hamster should prove useful in experiments dealing with insect transmission of these 3 agents and with the resistance of treated animals to infection.

14045

Factors Which Interfere with the Benzidine and Meyer's Tests for Blood in Milk and Urine.

MAX T. TRAINER. (Introduced by R. B. Gibson.)

From the Department of Hygiene and Preventive Medicine, The University of Iowa, College of Medicine, Iowa City, Iowa.

Introduction. A specimen of milk from a cow with mastitis, submitted to the State Hygienic Laboratory for examination, was tinged with blood and contained red corpuscles but was negative to Meyer's test.[1-3] A centrifuged portion was weakly positive and definitely so when the supernatant fluid was replaced by water. The benzidine test[4] gave similar irregularities.

The laboratory also had on record repeated tests on 2 cows which continued to shed red corpuscles in their milk throughout the lactation period. Their milk gave frequent negative tests for blood, becoming positive when the samples were centrifuged and the residues made up to volume with distilled water. Greene[5] has had similar experiences with clinical urine specimens whose microscopic examinations showed large numbers of red corpuscles but which were negative to the Meyer and benzidine tests.

The benzidine reagent may vary in its sensitivity;[6] false negatives may be obtained with small amounts of blood and the test is more sensitive to aqueous solutions than to naturally buffered biological fluids. False positive blood tests may be given by oxyhemoglobin, reduced hemoglobin, methemoglobin, carbon monoxide hemoglobin and acid hematin with both the benzidine and the less

1 Boas, I., *Deutsche Med. Wchnschr.*, 1915, **41**, 549.

2 *Merck Index*, ed. 5, Merck & Co., Inc., Rahway, N.J., 1940, 836.

3 Johannessen, A., *Ugesk. f. Laeger.*, 1921, **83**, 1613.

4 Hawk, P. B., and Bergeim, Olaf, *Practical Physiological Chemistry*, ed. 11, P. Blakiston's Son & Co., Philadelphia, 1937, 397.

5 Personal communication from Dr. J. A. Greene, Department of Internal Medicine, State University Hospital, Iowa City, Iowa.

6 Lyle, W. G., Curtman, L. J., and Marshall, J. T. W., *J. Biol. Chem.*, 1914, **19**, 445.