

countered twice. Hemorrhage from eroded vessels in the fibrous base of ulcers was the cause of death in 2 animals.

In swine, Wintrobe and associates have described atrophy and ulcers in the colon.⁴ They also reviewed signs of pantothenic acid deficiency in other species. No duodenal lesions occurred among their reported findings.

Whether the experimentally produced ulcers will acquire significance for the study of human ulcers remains to be seen. The possibility that the latter condition is caused directly by dietary pantothenic acid deficiency is remote considering the exceedingly wide distribution of this factor. To base a working hypothesis on these observations it would be

necessary to adduce evidence for abnormal paths of pantothenic acid metabolism. In rats, for instance, with adequate pantothenic acid in the diet but with prevention of intestinal synthesis of folic acid and biotin, a pantothenic acid deficiency in the tissues develops.⁵ It is relieved by feeding folic acid and biotin. Orienting studies are under way to test out possibilities of pantothenic acid being involved in the human duodenal ulcer. In the meantime, however, the findings herein reported should be of value in elucidating the various steps of the pathological process which go into the formation of a duodenal ulcer, particularly the early stages which are seldom seen in human material.

⁴ Wintrobe, M. M., Follis, R. H., Jr., Alcayaga, R., Paulson, M., and Humphreys, S., *Bull. Johns Hopkins Hosp.*, 1943, **73**, 313.

⁵ Wright, L. D., and Welch, A. D., *Science*, 1943, **97**, 426.

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17197. Neutralizing Antibody Against Viruses of the Encephalomyocarditis Group in the Sera of Wild Rats.

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The encephalomyocarditis (EMC),¹ MM,² Columbia-SK³ and Mengo encephalomyelitis⁴ viruses have been shown recently to be immunologically indistinguishable,^{5,6} hence, they are apparently strains of the same agent. The viruses of the EMC family are now known to have a world-wide distribution, occurring in North America,¹⁻³ the Philippines,⁷ and

Africa,⁴ but the natural history of the disease and sources of human infection are still obscure.

On at least two occasions viruses of the EMC group have been demonstrated to be pathogenic for man. Neutralizing antibodies against EMC virus have been shown to appear in the convalescent sera of a group of American soldiers who contracted a mild febrile illness colloquially designated as "Three-Day Fever"⁷ in Manila in 1945-46. In addition, acute encephalomyelitis caused by Mengo virus occurred in a laboratory worker at Entebbe, Africa.⁴

We were led to consider the possibility that the wild rat might serve as a reservoir for viruses of the EMC type for a number of reasons:

Mice, hamsters and cotton rats are highly susceptible and succumb to even small doses

¹ Helwig, F. C., and Schmidt, E. C. H., *Science*, 1945, **102**, 31.

² Jungeblut, C. W., and Dalldorf, G., *Am. J. Pub. Health*, 1943, **33**, 169.

³ Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

⁴ Dick, G. W. A., Best, A. M., Haddow, A. J., and Smithburn, K. C., *Lancet*, 1948, **2**, 286.

⁵ Warren, J., Smadel, J. E., and Russ, S. B., *J. Immunol.*, 1949, in press.

⁶ Dick, G. W. A., *J. Immunol.*, 1949, in press.

⁷ Smadel, J. E., and Warren, J., *J. Clin. Invest.*, 1947, **26**, 1197.

TABLE I.
Neutralizing Antibody Against EMC Virus in Sera of Wild Rats.*

Locale	No. tested	Result†		
		Negative	Positive	% positive
British Columbia	22	22	0	0
California	100	87	13	13.0
Florida	93	84	9	9.7
Georgia	57	51	6	10.5
Louisiana	9	9	0	0
Maryland	43	38	5	11.6
Mich. (Detroit)	11	11	0	0
Mississippi	49	6	43	87.0
New Mexico	6	6	0	0
North Carolina	2	2	0	0
South Carolina	21	21	0	0
Texas	23	16	7	30.5
Wash. (Seattle)	6	6	0	0

* Practically all the wild rats were *Rattus norvegicus*; some from California were *R. alexandrinus*, and all from South Carolina were cotton rats (*Sigmodon hispidus*).

† Sera containing sufficient antibody to protect against at least 100 minimal lethal doses of virus were considered positive, while those containing less than this amount were regarded as negative.

of virus injected peripherally.²⁻⁵

In contrast, the albino rat undergoes only an inapparent infection following a massive inoculum although considerable virus persists in the central nervous system^{3,5,8} and the circulation.⁹ The susceptibility of wild rats has not been determined but it was thought that it might be similar to the albino species.

One of the main foci of cases of Manila "Three-Day Fever" occurred in a camp located in a park which previously had been used by Japanese troops as a garbage dump. Although not mentioned in the original report of the outbreak, one patient whom we were able to interview recalled that rats were very numerous and cases of rat bite were occurring in this area.

Methods. The EMC, MM and Mengo viruses were maintained by intracerebral transfers in mice. The histories of the strains used in the present work were published in detail recently.⁵ Rodent sera were collected in various regions of the country and shipped to this laboratory;* approximately half were refrigerated during transit. Neutralization tests were performed in 12-14 g albino Swiss

mice using equal quantities of fresh infected mouse brain (appropriately diluted in distilled water) and undiluted serum. The virus-serum mixtures were incubated for 1 hour at 37.5°C after which 0.1 ml amounts of each mixture were inoculated intraperitoneally into each of 5 mice which were observed for 14 days. At the end of this time the neutralization index was calculated as the antilog of the difference of the LD 50% of the virus suspension in the control mixed with distilled water and in the test proper mixed with rat serum.

Experimental. Neutralization tests have been performed with sera from 442 wild rats (mainly *Rattus norvegicus* and *R. alexandrinus*) collected in various regions of the United States and Canada. Eighty-three of these had demonstrable antibody against EMC virus, *i.e.*, neutralized at least 100 M.L.D.'s of virus, and the majority of the positive sera possessed sufficient antibody to protect against 10,000 or more M.L.D.'s of virus.

The findings summarized in Table I show

⁸ Powell, H. M., Jamieson, W. A., and Culbertson, C. G., *Proc. Soc. Exp. Biol. and Med.*, 1948, **68**, 80.

⁹ Warren, J., and Russ, S. B., unpublished experiments.

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that the preponderance of sera examined came from rats caught in California, Florida, Georgia, Maryland and Mississippi, and that the proportion of positive sera among the rats from different regions varied considerably. For example, 43 of 49 specimens obtained from rural areas of Mississippi had antibody, whereas, only 6 of 57 from Georgia gave positive results. Certain counties within a state provided a greater proportion of positive sera than others. Thus, 11 of 44 samples from Orange County in southern California neutralized the virus, whereas, only 2 of 48 from San Diego County did so. Nine of the 93 sera examined from Florida gave positive results and 5 of these were among the five sera obtained from the town of Dania. It may be noted that it was in this area that the virus of encephalomyocarditis was originally recovered¹ from a chimpanzee held captive on an animal farm. Georgia supplied 6 positive sera and 5 of these were from rodents trapped in Wayne County. It would appear from the present data that the incidence of antibody against EMC virus is highest in rats caught in certain southern regions of the United States.

Twenty-two of the rat sera which were capable of neutralizing the EMC strain were also tested for their protective effect against the MM and Mengo strains. As was anticipated from earlier work the sera neutralized all 3 viruses to about the same extent.

EMC neutralization tests were performed on 69 sera obtained from various wild animals[†] which were caught in the same areas of California and Maryland that had yielded positive rat sera. (Table II). These included specimens from 4 gophers, 15 opossums, 9 rabbits, 18 squirrels, 2 chipmunks, 8 marmots, 2 meadow mice, 2 raccoons, 3 skunks and 6 domestic cats. None of these sera were capable of neutralizing even small amounts of

TABLE II.
Absence of Neutralizing Antibody Against EMC Virus in Sera of Rodents Other than the Rat.

Location	Species	No. tested	No. positive
Calif.	Gopher	4	0
	Opossum	13	0
	Rabbit	5	0
	Squirrel	18	0
	(<i>C. beecheyi</i>)		
Md. (Patuxent)	Chipmunk	2	0
	Marmot	8	0
	Meadow mouse	2	0
	Opossum	2	0
	Rabbit	4	0
	Raccoon	2	0
	Skunk	3	0
	Domestic cat	6	0

virus, *i.e.*, 50 M.L.D.'s.

Discussion. The EMC group of viruses appear to be ubiquitous, being widely distributed geographically and occurring in a number of host species. This should make one cautious in interpreting studies in which these viruses are apparently recovered from clinical material. Critical analysis of all the data is now required before one can assume that the agent found in passage animals bears an etiological relation to the disease in a given patient. Similarly, in view of present knowledge, "variant" strains encountered during serial transfer in animals such as the "high Lansing" mutant of poliomyelitis virus⁸ should be thoroughly investigated for possible relation to the EMC group.

Summary. Sera from an appreciable number of 442 wild rats trapped in several widely separated areas of the United States contained neutralizing antibodies against the EMC group of viruses. The incidence of positive sera varied considerably in different geographic regions with the highest rate (87%) occurring in rats from the state of Mississippi.

Similar serological studies on other species of small wild animals failed to provide evidence of the occurrence among them of natural infection with the EMC viruses.

[†] We are indebted to Drs. E. H. Lennette, State Department of Public Health, Berkeley, Calif., and J. A. Morris, Patuxent Research Refuge, Laurel, Md., for these sera.