phenacemide were employed as reference standards. 1. All 3 antimalarials were ineffective in protecting mice from maximal electroshock tonic extensor seizures, both after single oral doses and after 4 daily doses. 2. Hydroxychloroquine was effective against maximal metrazol seizures after a single oral dose. Quinacrine and chloroquine exhibited activity after 4 daily medications. 3. Quinacrine and chloroquine (low doses) enhanced psychomotor seizure duration while hydroxychloroquine and chloroquine (high doses) slightly reduced seizure duration. 4. Difficulties in predicting clinical efficacy from laboratory data are discussed.

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Antibodies to Mouse Hepatitis Viruses in Human Sera. (28928)

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Epidemiologic studies of viruses of the mouse hepatitis group have long been hampered by the lack of sensitive and specific serologic techniques. By using the continuous C3H mouse liver tissue culture cell line, clone NCTC 1469, which was originally cultivated by Hobbs *et al*(1) and applied to mouse hepatitis virus studies by Manaker *et al*(2), we have recently developed a plaque assay for many strains of this group(3). In addition, the fluids from infected cultures provide complement fixing (CF) antigens which are not only somewhat higher in titer than antigens prepared from infected suckling mouse liver and brain, but are often more sensitive for detection of antibody in mouse antisera (unpublished data); for example, the titer of MHV-S hyperimmune mouse antiserum was 1:128 when tested against 8 antigen units of tissue culture-grown MHV-S, but was only 1:32 with 8 units of MHV-S suckling mouse brain antigen.

This report describes the successful use of the plaque neutralization test and the tissue culture-produced CF antigens for demonstration of antibodies to mouse hepatitis viruses in human sera. The chief purpose is to present evidence for the specificity of the reac-

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TABLE I. Incidence of CF Antibody to A-59 Virus in Human Sera.

Geographic location	Type of population	Frequency of antibody* Children (under 9 yr)	(%)	Adults	(%)
Camp Lejeune and Parris Island	Recruits and military dependents	16/517	(3.1)	192/797	(24.1)
Great Lakes, Ill.	Recruits			22/80	(27.5)
Washington, D. C.	General population	12/287	(4.2)	78/459	(17.0)
Total		28/804	(3.5)	292/1338	(21.8)

* No. persons positive (3 to 4+ CF reaction of 1:8 serum dilution tested vs. 8 to 16 antigen units of A-59 virus)/No. tested. When serial specimens were tested only the last serum is included in the tabulations.

tions observed; epidemiologic studies will be presented elsewhere.

Materials and methods. Complement fixation tests were done by the micro procedure (4,5) using 2 full units of complement and overnight incubation at 4°C. Antigens consisted of infected NCTC 1469 cells in culture supernates, and were used undiluted or at a 1:2 dilution, corresponding to 8 to 16 antigen units as determined with homotypic hyperimmune mouse serum. Preliminary studies indicated that the A-59 antigen was more highly reactive with human sera than were several other MHV strains, in that both the frequency of reaction and the titers obtained were greater. Consequently, this antigen was used for the great majority of tests. Screening tests were done with 1:8 serum dilution.

Plaque neutralization tests were done primarily with strains A-59(2), MHV-S(6), and a recent isolate, M-CDF, recovered from feces of normal CDF_1/N mice. These strains were selected primarily because of the distinctness of plaques. The tests were carried out in 60 mm plastic Petri dish cultures of clone NCTC 1469. Serum (heated 56° 30 min.) was diluted in Eagle's basal medium (EBM) with 20% veal infusion broth; virus was diluted in EBM with 10% chicken serum. Equal parts of serum dilution and of a virus dilution containing about 100 pfu per 0.1 ml were mixed and held at 37°C for 1 hour. The serum-virus mixtures were then diluted with an equal volume of EBM with 20% veal infusion broth, and 0.4 ml of the mixture was inoculated onto the cell sheet of each of 2 dishes from which the fluid had been decanted. After an adsorption period of 45 minutes at room temperature, the dishes were overlaid with 5 ml of 0.9% Noble agar containing 5% chicken serum in Mixture 199.

After 48 hours' incubation at 36°C in a humidified atmosphere containing 5% CO_2 in air, the cultures were stained for 2 hours with 2 ml of 1:20,000 neutral red solution. The definitive plaque count was made on the following day. For screening tests, sera were used at 1:10 dilution, and the results tabulated as percentage reduction in plaque number relative to virus control. For quantitation of antibody, serial 4-fold serum dilutions were tested, and the percentage plaque reduction plotted on probit paper; the serum dilution graphically estimated to produce 67% plaque reduction was taken as the titer of the serum.

The majority of sera were from a longitudinal study of U. S. Marine recruits undergoing recruit training at Parris Island, S. C., followed by advanced infantry training at Camp Lejeune, N. C. In addition, sera were tested from children of military personnel at Camp Lejeune, from recruits at Great Lakes Naval Training Station, and from general population groups in Washington, D. C. The majority of paired sera were obtained at 3 to 4 week intervals.

Results. Positive complement fixation reactions were encountered in all population groups studied; the incidence is given in Table I. The frequency of reactions was similar in the different geographic areas, and was greater in adults than in young children.

TABLE II. Correlation of Mouse Hepatitis Virus CF and Neutralizing Antibody in Human Sera.

Age group	Virus strain used in neutralization test	Frequency* of plaque neutralization by 1:10 serum dilution vs. indicated virus, in relation to degree of CF reaction at 1:8					
		Positive CF (3 to 4+)		Partial CF (2+)		Negative CF (0 to 1+)	
			(%)		(%)		(%)
Adults	A-59	31/32	(97)	4/5		16/37	(43)
	MHV-S	30/47	(64)	8/13	(62)	10/87	(14)
	M-CDF	8/9	(89)	1/1		9/22	(41)
	Antibody for one or more of above	46/49	(94)	12/14	(86)	31/91	(34)
Children	A-59	5/9		—		1/7	
	MHV-S	2/7		0/1		0/6	
	M-CDF	7/7		—		1/7	
	Antibody for one or more of above	9/10		0/1		1/8	

* No. of sera positive in neutralization test/No. tested.

Of 544 paired sera from Marine recruits reporting for various reasons to the Camp Lejeune dispensary during January to March of various years, 65 (12%) showed 4-fold or greater rises in CF titer to A-59 virus. The rises occurred during each of 3 winters of this study, the frequency of rises ranging from 5.7% to 15.7% in the different years. In this population, sera taken during other seasons rarely showed rises. Rising titers were also observed in similar sera obtained at Parris Island, the Great Lakes station, and in Washington, D. C. The majority of rises were conversions from negative serology in the acute phase serum.

Sera reacting in CF with viral antigens were consistently negative against control NCTC 1469 cell antigens. Many of the serum pairs from the Marine and general population groups were also tested for CF antibody responses to respiratory agents, including adenoviruses, influenza A and B, parainfluenza 1, 2, 3, and 4 viruses, respiratory syncytial virus, and *Mycoplasma pneumoniae*. There was no association between rises to MHV and any of the tested agents, although the "outbreaks" of MHV rises occurred during periods when adenoviruses were epidemic. In addition, selected pairs with rises to MHV showed no CF antibody responses to reovirus types 1, 2, and 3; and paired sera of 3 persons with CF rises to reovirus antigen showed no rises to A-59. One of 14 serum pairs with rises to A-59 showed CF antibody response to measles antigen; 6 of the pairs had measles antibody in equal titer in both sera, and 7 were negative in both. In similar tests with SV 5 (parainfluenza 2 simiae) and mumps antigens, one of 9 paired sera showed a rise to both antigens, one was positive for mumps in equal titer in both sera, while the remainder were negative for both antibodies.

None of the persons with MHV antibody rises had clinical disease suggesting hepatitis, and of a small group of sera from patients with infectious hepatitis, few showed CF antibody to the A-59 virus, and none showed rising or high titers. Preliminary analyses of tests of sera from controlled illness studies at Camp Lejeune suggest no relationship of response to MHV to upper respiratory disease.

Identification of the CF reactive material as a specific antiviral antibody is provided by a number of lines of evidence. The most definitive evidence is the close correlation found between results of CF and neutralizing antibody tests. One hundred and seventy-three sera tested in CF against A-59 and/or MHV-S were tested in the plaque neutralization test against one or more MHV strains (Table II). The overwhelming majority of CF positive sera neutralized at least one and usually all of the tested strains.

For each of the viruses, the correlation of neutralization result with CF reactivity was highly significant. The MHV-S virus was

TABLE III. Correlation of CF and Neutralizing Antibody Rises to Mouse Hepatitis Viruses in Human Serum Pairs.

Virus strain used in neutralization test	Frequency* of neutralizing antibody rise in serum pairs with indicated CF antibody response to A-59			
	≥ 4-fold CF rise	(%)	No CF rise	(%)
A-59	14/31	(45)	0/5	
MHV-S	6/29	(21)	0/16	
M-CDF	12/18	(67)	1/8	
Antibody rise to one or more of above	21/34	(62)	1/16	(6)

* No. of paired sera showing ≥ 4-fold neutralizing antibody rise/No. of pairs tested.

less often neutralized than the A-59 and M-CDF strains.

Results of tests of paired sera showed a similar correlation (Table III). Fifty paired sera from various population studies were tested for plaque neutralizing antibody rises to one or more of the 3 strains; the majority of pairs showing CF antibody rise also had rises in neutralizing antibody titer to one or more strains, while paired sera without CF rise rarely showed neutralization rise. None of the pairs showed falling neutralization titers.

Paired sera of 3 recruits demonstrating an antibody response were studied more intensively for specificity of this response and for degree of reactivity with various strains of MHV (Table IV). The results are completely compatible with a specific antibody response to antigens shared by many viruses of this group. The absence of CF reaction with the tissue antigens is somewhat surprising, but is probably due to their lesser reactivity as mentioned above.

Three lines of evidence indicate that the antibody was not a heterospecific antibody against mouse or liver protein. First, the positive sera did not react with control tissue and tissue culture antigens (Table IV). Second, absorption of serum with concentrated suspensions of clone NCTC 1469 tissue culture cells did not affect either the CF or plaque neutralization titers against A-59 and MHV-S viruses, respectively. Third, comparative neutralization tests in which 1:10 virus and 1:40 serum dilutions were mixed, and the mixture diluted 1:100 before

TABLE IV. Antibody Responses of Three Marine Recruits to Various Strains of Mouse Hepatitis Virus.

Serologic test	Virus strain	Serum (name and date) Bur. 2-24-62	Bur. 3-26-62	Eng. 2-24-62	Eng. 3-26-62	Aub. 2-2-62	Aub. 3-2-62
		Serum antibody titer					
CF with tissue culture antigens	A-59	0*	16	0	32	0	32
	M-CDF	0	32	0	32	0	32
	MHV-S	0	8	0	8	0	8
	MHV-1	0	4	0	0	0	(8)†
	MHV-3	0	8	0	16	0	8
	JHM	0	0	0	0	0	0
	Control	0	0	0	0	0	0
CF with mouse brain antigens	A-59	0	0	0	0	0	0
	MHV-S	0	0	0	0	0	0
	Control	0	0	0	0	0	0
Plaque neutralization	A-59	6	125	11	510	9	97
	M-CDF	5	470	13	385	7	600
	MHV-S	5	240	13	400	8	82
	MHV-1			8	540		
	JHM	6	136				
	M-N‡			2	128		
		Neutralization index of 1:10 serum dilution					
Suckling mouse neutralization	MHV-S	13	590	35	650		

* 0 = less than 1:4. † Partial CF reaction at 1:8.
‡ A strain of MHV recovered from feces of normal Swiss mice of Colony "N"(6).

plating, gave plaque reduction comparable to that obtained when 1:1000 virus dilution was mixed with 1:40 serum dilution, and the mixture plated without further dilution. This result would indicate that the plaque reduction was not produced by a serum component which alters the adsorptive capacities of the tissue culture cells.

Discussion. The agent responsible for the MHV antibodies in human sera is not known. Three possibilities are: i) a known virus of man; ii) mouse hepatitis strains acquired by contact with mouse feces(6); and iii) an undescribed virus of man which is generically related to the mouse hepatitis viruses.

The first of these seems quite unlikely. The mouse hepatitis viruses are chloroform-sensitive (unpublished data) agents of roughly 90 mμ diameter(7,8), which appear to form in the cytoplasm(8,9). They do not hemagglutinate or give positive hemadsorption, and do not produce cytopathogenicity in monkey or human kidney cell cultures. Thus, their properties differentiate them from most known viruses of humans; myxovirus-like agents which produce giant cell cytopathic effects, such as measles and respiratory syncytial viruses, share some of these characteristics, but the serologic data clearly exclude these two as the source of the antibodies.

The second possibility cannot be excluded, but seems somewhat unlikely on epidemiological grounds. The seasonal occurrence of the increase in antibody at Camp Lejeune could be due to rodent infestation of barracks or kitchens during winter; but few rodents were noted, and the training at Camp Lejeune consists in large part of field exercises, where rodent contact in winter would be minimal. Also, no MHV antibody was found in sera of 30 wild mice trapped at Camp Lejeune, and no virus was detected in intestine suspensions of 19 of the mice (unpublished data). In addition, our laboratory animal caretakers, with intensive exposure to naturally and artificially infected mice, have not shown antibody responses.

The third possibility, a virus of the MHV group maintained in human populations, seems somewhat more likely, but is not supported by direct data. Viruses of the MHV group have not been recovered from species other than mice, and our attempts to recover such viruses from throat and anal swabs of recruits with MHV antibody rises have been unsuccessful. However, adult laboratory rats, with the exception of germ-free animals, show very high rates of CF and plaque-neutralizing antibody to MHV (unpublished data). By study of the rat infections it may be possible to clarify whether species-specific strains of viruses related to MHV exist.

The possibility was considered that a component in a vaccine administered to recruits was responsible for their responses, but the majority of rises occurred at Camp Lejeune, more than 2 months after the intensive phase of the recruits' immunization schedule was completed. In addition, this hypothesis does not explain the seasonal occurrence of antibody rises.

Summary. Human sera frequently react with mouse hepatitis viruses in CF and neutralization tests; available evidence indicates that the reacting factor is a specific antiviral antibody. Antibody responses were observed in several population groups, particularly in Marine recruits during winter. The agent responsible for the antibody is not known.

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