TABLE I.
Results of Administration of Icterogenic Serum (Infectious Hepatitis) to Human Volunteers.

Group	Subjects	Route	Number of volunteers		Incubation period, days
			Inoculated	Jaundiced	
A	Normal subjects	Par.*	7	2	24, 31
B	Inoculated unsuccessfully 6 months before with agent of homologous serum jaundice	"	1	1	21
C	Convalescent 6 months from homologous serum jaundice	"	3	3	20, 20, 25
D	Normal subjects	Oral	4	3	23, 24, 34
E	Fed unsuccessfully 6 months before with agent of homologous serum jaundice	"	1	1	29

* Par. = Parenteral inoculation.

jaundice after 20, 20, and 25 days respectively. It is also of note that the incubation periods in these experimental cases have ranged from 20-34 (average 24) days, with no appreciable difference between those inoculated parenterally and those fed equal amounts of the same infectious agent.

Summary. Previous experimental infection (or inoculation without infection) with a strain of *homologous serum jaundice* obtained in the Middle East failed to protect 5 human volunteers against subsequent experimental infection induced 6 months later with a strain of *infectious hepatitis* also obtained in the Middle East.

These results are in contrast to those found by Oliphant, who has described immunity in human subjects convalescent 6-19 months from homologous serum jaundice, when inoculated with serum from acute cases of infectious hepatitis.

Using a strain of the *naturally occurring disease,* infectious hepatitis, no appreciable difference in incubation period was observed in experimental cases when equal amounts of the same infectious agent were inoculated parenterally or fed. Incubation periods ranged from 20-34 (average 24) days.

15014

Immunologic Relationships of MM, Lansing Poliomyelitis and Mouse Encephalomyelitis Viruses.

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A number of viruses have the physical properties of poliomyelitis virus and induce poliomyelitic lesions in rodents. Those discovered by Theiler are known as mouse encephalomyelitis virus.[1] The others are thought to be strains of human poliomyelitis which have been adapted to rodents.[2-6] Both groups include strains which differ clinically and in infectivity from human poliomyelitis and spontaneous mouse encephalomyelitis.† Largely because of this Jungeblut's suggestion[7] that

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1 Theiler, M., *J. Exp. Med.*, 1937, **65**, 705; *Medicine,* 1941, **20**, 443.

2 Armstrong, C., *Pub. Health Rep., U.S.P.H.S.,* 1939, **54**, 1719.

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

4 Toomey, J. A., and Takacs, W. S., PROC. SOC.

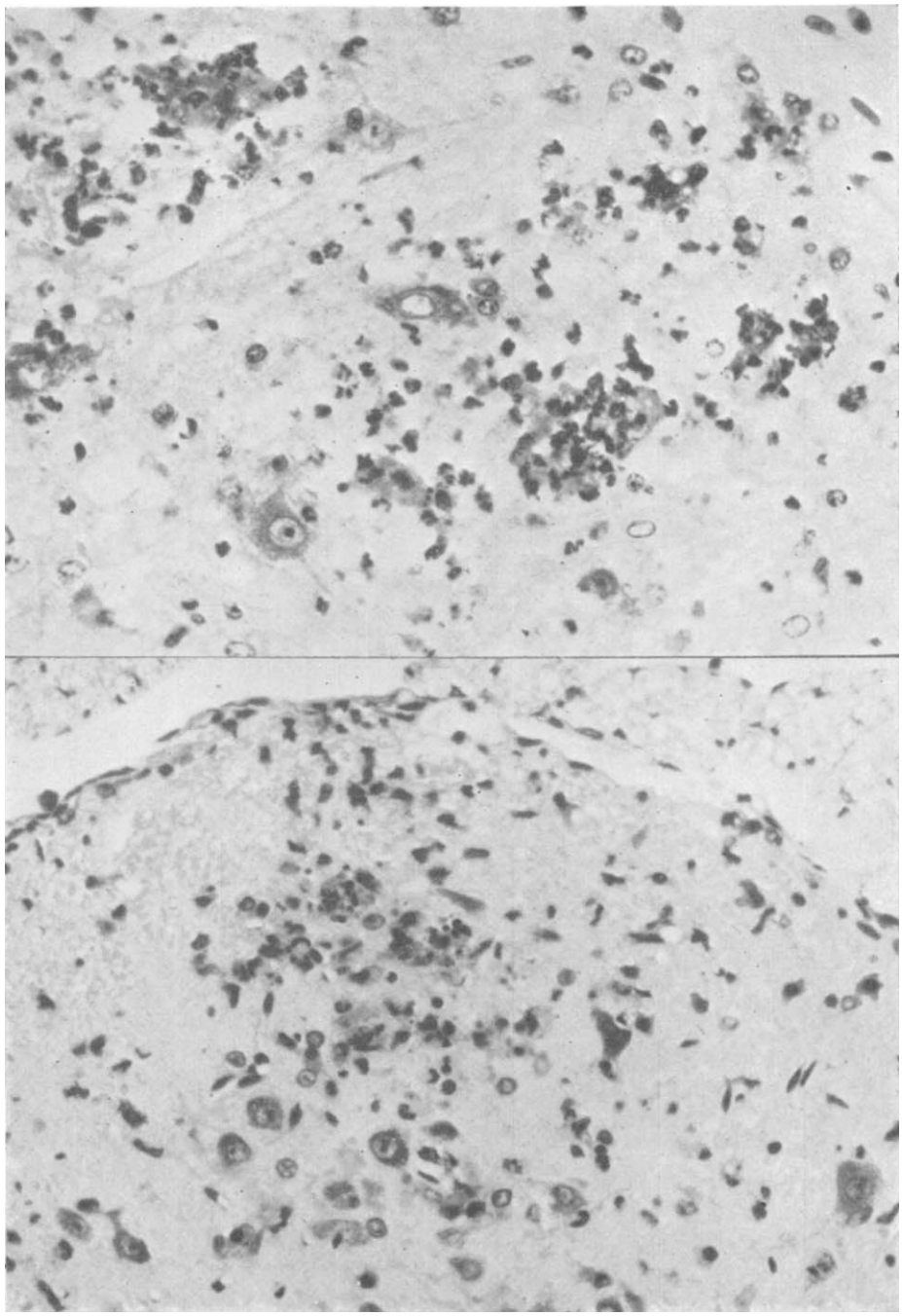

FIG. 1.

Lesions of anterior horns of hamsters following intraperitoneal injection of MM virus. The upper photograph is of the thoracic cord and shows several areas of neuronophagia with polyblasts and polymorphonuclears. The lower photograph shows a minute lesion in the sacral cord of another animal. Both specimens were prepared from hamsters which had been paralyzed for more than a week.

TABLE I.
Neutralization of MM Virus by Various Sera.

Serum	A—Mouse Tests Days on which mice died or were paralyzed Virus dilutions				
	10-2	10-4	10-6	10-8	10-10
MM convalescent hamster	3,5	5,0	0,0	0,0	0,0
GDVII convalescent hamster	2,2	2,3	2,2	2,3	3,3
McG hyperimmune rabbit*	2,2	2,2	2,3	3,3	2,3
Lansing hyperimmune rabbit	3,5	3,4	0,0	0,0	0,0
MM hyperimmune rabbit	3,3	5,5	0,0	0,5	0,0
Normal rabbit	1,2	2,2	3,3	3,3	2,3
Normal hamster	2,2	2,2	2,2	2,3	3,3

* A strain of mouse encephalomyelitis virus (see Table II).
Mixtures injected intracerebrally in 3-week-old Swiss mice (0.03 cc).

	B—Hamster Tests Virus dilutions					
	10-3			10-6		
	No. tested	No. paral.	No. died	No. tested	No. paral.	No. died
MM convalescent hamster	6	0	0	6	0	0
Lansing hyperimmune rabbit	6	4	0	6	0	0
Lansing convalescent hamster	6	6	2	6	6	1
Normal rabbit	6	5	4	6	5	1

Mixtures injected intraperitoneally in young animals (0.1 cc).

all be included in a "poliomyelitis group" has not been adopted.

MM virus, apparently derived from a human case of poliomyelitis by hamster passage,[5] is highly infectious and is present in large amounts in diseased animals. It is infective by peripheral as well as central routes. On the other hand the lesions (Fig. 1) seem to us to be typical of poliomyelitis and the physical properties[5] are similar to those of human strains. MM virus therefore renews the problem of terminology and taxonomy and the present report is submitted as a contribution to that problem as well as a further documentation of the MM virus.

Virus Neutralization. The sera of convalescent hamsters effectively neutralize MM virus. The antibodies appear within 10 days and rapidly increase. Sera collected during the 4th week usually neutralize more than a million infective doses.

The sera of hamsters convalescent from Lansing poliomyelitis or weak strains of mouse encephalomyelitis virus do not contain appreciable amounts of antibody but potent antisera can be prepared by repeatedly injecting rabbits with mouse brain suspensions from diseased animals. The sera used in the present work were prepared in this manner. Eight injections were given at 3- or 4-day intervals. The first 4 injections consisted of 5 cc of a 10% suspension of mouse brain injected intraperitoneally while the last 4 included, in addition, 0.5 cc of 0.1% suspensions injected intravenously. The final intravenous injection was of 10% suspension.

Using such sera and incubating equal parts of them and various dilutions of virus for 2 hours at room temperature it has been found that Lansing poliomyelitis hyperimmune rabbit serum neutralizes MM virus very

EXP. BIOL. AND MED., 1940, **45**, 364; 1941, **46**, 22, 319.

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

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

† Olitsky (Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 77) has recently discussed the significance of these differences.

7 Jungeblut, C. W., *Am. J. Pub. Health*, 1943, **33**, 1227.

TABLE II.
Cross Immunity Tests in Mice Previously Injected Intracerebrally with Mouse Encephalomyelitis Virus (MeG Strain).

Test virus	Day on which the mice died or were paralyzed	
	Experimental group	Control group
Theiler's	11,11,12,19,0,0,0	11,11,12,12,13,19,19
(TO 0.03 cc IC)	0,0,0,0,0,0,0,0	23,23,23,29,0,0,0
MM (IP)		
10-1	3,4,5,5,5,6	4,4,4,4,5,7
10-3	4,6,10,10	4,6,9,10
10-5	5,5,0	5,0,0
MM (IC)*		
10-3	7,7	7,7
10-6	7,7	7,0

* Large adult Swiss mice. 10-6 is end point in mice of this age and strain. All groups of same age and strain. All tested mice non-paralyzed convalescents. Interval between injections 28 days.

TABLE III.
Cross Immunity Tests in Hamsters Previously Injected Intraperitoneally with MM Virus and Challenged with Lansing Poliomyelitis Virus Injected Intracerebrally.

Response to MM virus	Number	Response to Lansing virus
Experiment 1. Interval 14 days.		
Paralyzed but completely recovered	18	0 paralyzed
Not paralyzed	18	6 "
Normal controls	19	5 "
Experiment 2. Interval 32 days.		
Paralyzed but completely recovered	7	0 "
Paralyzed. Slight residual paralysis in hind legs*	11	No further paralysis
Not paralyzed	15	0 paralyzed
Normal controls	23	6 "

(In both experiments animals of the same age were used throughout.)
* Lansing poliomyelitis affects forelegs more frequently than hind legs. In 4 of the 6 controls paralysis was limited to the forelegs.

effectively while mouse encephalomyelitis hyperimmune rabbit serum does not (Table I). The latter effectively neutralizes homologous virus in 10% suspensions of mouse brain.

Tests have also been made of these antisera against Lansing virus including trials in which the antisera were mixed with equal amounts of glycerin and the virus containing nervous tissue stored in the mixtures for as long as 3 weeks; the virus content was then determined by mouse inoculation. The number of paralyzed mice can be reduced from 73% to 53% by using either Lansing hyperimmune rabbit serum or MM convalescent hamster or hyperimmune rabbit serum. The mice which receive the test sera rather than normal rabbit serum also exhibit delayed onset of paralysis. These tests have been repeated by Jungeblut, using our sera, with similar results.[8] It is interesting to note that convalescent serum from A.D., one of the patients of the 1942 epidemic from which the MM virus originated, likewise neutralizes Lansing virus to this degree.

Cross Immunity Tests. Cross immunity tests were extensively used in classifying mouse encephalomyelitis virus strains.[1] Subsequent work has detracted from the reliability of such tests since interferences are

[8] Jungeblut, C. W., personal communication.

TABLE IV.
Results of Complement-Fixation Tests.

Serum	Antigen		
	MM	GDVII	SK (Ret. 3)
McG rabbit	—	2+ (1:6)	2+ (1:24)
Lansing rabbit	—	2+ (1:6)	3+ (1:6)
GDVII rabbit	4+ (1:24)	4+ (1:12) 1+ (1:24)	3+ (1:24)
SK rabbit	—	1+ (1:6)	4+ (1:12)
MM rabbit	4+ (1:48)	4+ (1:48)	4+ (1:48)
Normal rabbit	—	—	—

The figures in parentheses indicate dilution of antigen. Sera all prepared by hyperimmunization of rabbits.

known to occur in experimental poliomyelitis.[9] We have not devised a wholly satisfactory means of separating cross immunity from interference in the case of these viruses. The chief difficulty is that if the interval between injections be greatly prolonged the test animals are usually spontaneously immune because of age.

Some of the réactions[10] are clearly due to interference while others may represent cross immunity. In those summarized in Tables II and III the likelihood of interference has been minimized in the first case by demonstrating different responses to 2 simultaneously injected challenging viruses and in the second case by using as the initial virus the MM strain which does not persist long in the hamster. In this experiment representative paralyzed and non-paralyzed animals were sacrificed and found free of transmissible infection before the challenging virus was injected.

The results of these experiments support the neutralization tests in indicating a relationship between MM and Lansing viruses but no relationship between MM and mouse encephalomyelitis viruses.

Complement-Fixation Tests. The viruses have also been compared by complement-fixing tests. The antigens were prepared from recently paralyzed hamster brains by the method of Casals and Palacios.[11] The results are shown in Table IV and are difficult to correlate with the neutralizing and cross immunity tests in that they show no reaction between MM antigen and Lansing hyperimmune rabbit serum although the latter does react with both GDVII mouse encephalomyelitis and SK viruses. On the other hand GDVII rabbit serum reacts well with MM antigen. We have been unable to prepare satisfactory Lansing antigen, probably because of the low titer of virus in paralyzed hamsters.

Discussion. A comprehensive discussion seems unwarranted at this time because the basic problem should be decided by a review of all of the applicable evidence. The results described give substantial support to the view that MM virus is a strain of poliomyelitis. If this is true it is the first strain to be adapted to rodents by hamster passage, an adaptation we have failed to repeat in several later attempts with other strains. The results also support Jungeblut's conception of a group of poliomyelitis viruses including some strains of mouse encephalomyelitis. We believe the results of the cross immunity tests to be indecisive in view of the possibility of interference but the neutralization tests yield evidence which seems trustworthy. This is particularly true because the rabbit sera used have shown a high degree of specificity. There is no evidence of broadened reactivity such as is true of anti-influenza hyperimmune rabbit sera.[12] Sera from rabbits immunized by injecting GDVII virus have been found to

9 Dalldorf, G., Douglass, M., and Robinson, H. E., *Science*, 1937, **85**, 184; Dalldorf, G., and Douglass, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 294; Jungeblut, C. W., *J. Exp. Med.*, 1945, **81**, 275.

10 Dalldorf, G., and Whitney, E., *Science*, 1943, **98**, 477.

11 Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

neutralize both MM and SK viruses but the reverse reactions do not occur.[13]

Theiler[1] reported the absence of serologic relationship between mouse encephalomyelitis and Lansing poliomyelitis but relative resistance of mice paralyzed by mouse encephalomyelitis to Lansing virus. He considered the resistance non-specific because it seemed to depend on the actual presence of paralysis. We have confirmed these results. Mice seem less suitable than hamsters for experiments of the kind.

The usefulness of MM virus as a laboratory tool deserves mention. The disease in intraperitoneally injected hamsters is remarkably like human poliomyelitis. Most of the animals survive and the majority of the survivors completely recover the use of their extremities. Antibodies develop rapidly. The virus is stable in glycerin for at least 17 months. Finally MM virus seems of especial interest by being intermediate between Lansing poliomyelitis and mouse encephalomyelitis and therefore especially adapted to taxonomic studies.

Conclusions. Neutralization and cross immunity tests indicate a relationship between MM and Lansing poliomyelitis but no relationship between MM and TO (Theiler original or low virulence strains) mouse encephalomyelitis. Complement-fixation tests relate MM virus and the GDVII strain of Theiler's virus. The results support the view that a group of poliomyelitis viruses exists which may be related by serologic tests.

[12] Francis, T., Jr., and Magill, T. P., *Brit. J. Exp. Path.*, 1938, **19**, 284.

[13] Jungeblut, C. W., *Am. J. Pub. Health*, 1944, **34**, 259.

15015

Further Investigations on the Recovery of Inhibited Conditioned Reactions.*

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It has been shown in several preceding investigations[1] that conditioned reactions (c.r.) which had been inhibited in rats by lack of reinforcement can be re-established without further training process by subjecting the animals to either insulin coma or electrically induced convulsions. In order to gain insight into the neurophysiological processes underlying this effect it was thought desirable to extend the investigations to animals in which more than one c.r. had been established.

Method. Successful experiments were carried out on 10 rats. In some of these 2 to 3 c.r.'s were established in succession. However, no new c.r. was formed until the preceding c.r. had been inhibited by lack of reinforcement. A doorbell, a sound of 250 vibrations per second, and a light served as conditioned stimuli (c.s.). A shock applied to the grid on which the rat was standing was the unconditioned stimulus and the c.r. was an escape reaction as described in the previous papers.

In the second group of animals an attempt was made to maintain one conditioned reaction at about 100% and to inhibit another conditioned reaction without influencing the first. Such animals were used in order to decide the question as to whether the action of insulin coma and electrically induced convulsions is

* Aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Gellhorn, E., Kessler, M., and Minatoya, H., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 260; Gellhorn, E., and Minatoya, H., *J. Neurophysiol.*, 1943, **6**, 161; Kessler, M., and Gellhorn, E., *Am. J. Psychiat.*, 1943, **99**, 687; Gellhorn, E., *J. Lancet*, 1943, **63**, 307.