In the previous work(2), sera which were tested for Mo agglutinins were preserved with Merthiolate. To determine whether Merthiolate had a destructive effect on this agglutinin, 16 sera were collected and within 24 hours were inactivated and divided into 2 portions, to one of which Merthiolate was added as described previously. These sera were then stored in a refrigerator for periods ranging from 6 to 19 days following which the merthiolated and unmerthiolated sera were tested simultaneously. The difference between titers of serum with and without Merthiolate never varied more than one tube. In 5 cases, the titer of merthiolated serum was ½ tube lower than the serum without Merthiolate. In one case, the titer of merthiolated serum was one tube higher and in another case ½ tube higher than the serum without Merthiolate.

Discussion of results. The Mo agglutinin, a naturally occurring human hetero-hemagglutinin for mouse erythrocytes, was found in all the 400 sera tested in this investigation. The previously reported(2) failure to detect agglutinins in sera of some human subjects was undoubtedly due to the fact that the method did not detect agglutinins in sera with low titers. Waller(3) has shown that Merthiolate, in small concentrations, inhibits the action of anti-Rh agglutinins. If this were true of the Mo agglutinin, it would constitute additional reason for the large number of sera which were reported as not containing the agglutinin in the previous investigation. Results obtained in the merthiolated series of sera seemed to indicate that there might be a small degree of inactivation of the Mo agglutinin by Merthiolate. However, this inactivation was not of sufficient degree to account for the results obtained previously. Age, sex, color or major blood groups were not related to the degree of agglutination exhibited by the sera tested. Gorer(1) has used what, in all probability, is this agglutinin in detecting antigenic similarities and differences in various strains of mice. Thus this agglutinin was used to detect what he has called Antigen III. The question of whether the Mo agglutinin may have some significance comparable to the heterophilic antibody found in infectious mononucleosis should be investigated.

Summary. The Mo agglutinin (a human agglutinin for mouse erythrocytes) was found to be present in 400 human sera tested. The failure of previous attempts to demonstrate the agglutinin in all sera was probably due to the fact that the method used was not sufficiently sensitive. Merthiolate has little or no inactivating effect on the Mo agglutinin. The significance of the variation in amount of Mo antibody in human sera has not been determined.

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Neurotropic Viruses in Extraneural Tissues. III. MM Virus in Human and Murine Testicular Tissues.* (18439)

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In previous papers of this series(1,2), MM virus has been shown to multiply in the following tissues of hamsters and/or mice: soft tissue of the hind foot, skeletal muscle, skin, pharyngeal wall, urinary bladder wall, and embryonic tissues. All of the specimens of tissue in which virus was shown to multiply contained either smooth or striated muscle.

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From these studies and the investigations of others it appears that MM and the closely related viruses, Columbia SK and EMC, are characterized by pronounced myotropism as well as by their well known neurotropism.

In attempts to determine whether MM virus will multiply in extraneural cells other than muscle, we have used principally lymph nodes and testes. Up to the present, inconclusive results have been obtained with lymph nodes. In this paper it will be shown that MM virus multiplied in human and murine testicular tissue *in vitro*. Attempts to demonstrate multiplication in murine testes inoculated *in situ* gave less conclusive results.

Experimental methods. The virus employed was from the same source, and the experimental animals were from the same colony as described in previous studies(1,2). Tissue culture methods were the same as those described in previous papers(2,3). Approximately 0.04 ml of minced tissue was incubated in 4 ml of a fluid medium composed of bovine serum ultrafiltrate diluted with 3 volumes of Hank's solution with 100 units of penicillin G and 100 μg of streptomycin or dihydrostreptomycin per ml. The flasks were flushed with 5% CO_2 in air, closed with rubber stoppers, and incubated at 36° to 37°C. After each 3 or 4 days' incubation, 3.6 ml of medium were removed from each flask and replaced by an equal volume of fresh medium to provide a continuous supply of nutriments. All cultures were prepared in triplicate and tests for virus were made using pooled fluid from all 3 flasks. Virus content of specimens was determined by preparing serial 10-fold dilutions in Ringer's solution containing 0.5% glucose and injecting 3 mice approximately 1 month old intracranially with 0.03 ml of each dilution. All mice that survived the first 24 hours and died within 7 days were considered to have died of infection with MM virus. Checks for bacterial contamination of the inoculum were made routinely on Proteose peptone No. 3 agar, tryptose agar or veal infusion agar. During intervals of storage specimens were kept in sealed glass tubes in a dry ice chest.

Results of experiments on multiplication of MM virus in testicular tissues of mice. In 2 experiments a total of 13 testes of mice approximately 12 weeks old were inoculated with between 100 and 1000 minimal infective doses (m.i.d.) of MM virus and subsequently removed at 2, 7, 24, or 48 hours and assayed for virus. Virus was present at 24 hours in titers of 10^{-2} to 10^{-3}; at 48 hours, 1 testis gave similar results but 2 of the 3 testes removed at this time contained no detectable virus. These results suggested that the virus might have multiplied locally in some but not all testes. Whether the multiplication occurred in muscle cells of the scrotum, the cremaster, sustentaculum, or in nonmuscular tissues could not be determined. In experiments on propagation of MM virus *in vitro* in mouse testicular tissue, white mice approximately 7 months old were used. Virus with an LD_{50} of $10^{-5.25}$ was added to the tissue in each flask in an amount sufficient to give a 10^{-5} dilution. Tests at 3, 6, 10 and 13 days showed that the largest amount of virus was present at 3 days; no virus was detected at 10 and 13 days.

An experiment was carried out in which 9 serial subcultures of virus were made with incubation periods of 3 or 4 days in each tissue and occasional periods of storage of fluid medium in the frozen state. Tests for virus in medium at the end of each 3- or 4-day interval were always positive. The calculated dilution of the original inoculum in the 9th serial passage was 10^{-13}. Fluid medium from this culture killed all 3 mice injected with the 10^{-2} dilution and 1 of 3 mice injected with the 10^{-3} dilution. It is therefore evident that MM virus multiplied extensively in this series.

In checking the identity of the virus present in the last tissue culture of the series, it was shown to be resistant to ether treatment overnight in the refrigerator and to cause paralytic infection after intramuscular inoculation of mice and hamsters. Bacteriological tests were negative.

Results of experiments on multiplication of

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TABLE I. Tests for MM Virus in Tissue Cultures of Human Testes.

Tissue culture*	Culture age, days	Tissue age, days	Inoculum age, days	Calculated dilution of inoculum	Fate of mice inj. with dil. of tissue culture fluid				
					10^{-0}	10^{-1}	10^{-2}	10^{-3}	
T_1F_1	1	1	1	10^{-5}	3 4 4‡	s s s	s s s	s s s	
	2	2	2	10^{-5}	s s s	s s s	s s s	s s s	
	3	3	3	10^{-5}	(4) 8 s	s s s	s s s	s s s	
T_1F_2	2	5	5	10^{-6}	3 3 4	3 3 3	3 s s		
	3	6	6	10^{-6}	3 4 4	3 3 4	5 s s		
†	4	7	7	10^{-6}	2 3 3	(1) 3 3	3 9 s		
T_1F_3	3	10	10	10^{-7}	3 3 4	3 4 s	s s s		
T_2F_1	1	9	8	10^{-7}	4 4 s	(12) s s	s s s	s s s	
	3	11	10	10^{-7}	3 4 5	4 7 s	s s s		
†T_2F_2	4	15	14	10^{-8}	3 3 5	3 4 4	3 s s		
T_2F_3	4	19	18	10^{-9}	(1) s s	s s s	s s s		
T_3F_1	4	4	18	10^{-9}	s s s	5 s s	(14) s s		
†T_3F_2	4	8	22	10^{-10}	3 3 3	3 3 3	3K s s		
T_4F_1	4	4	26	10^{-11}	3 3 3	3 5 s	4 s s	s s s	July 29
T_4F_2	4	8	30	10^{-12}	2 2K 3	2 3 3	3 4 4	s s s	
T_4F_3	4	12	34	10^{-13}	3 4 5	3 5 6	s s s	s s s	

* T_1 = first tissue exposed to virus. T_2 = second set of tissues exposed to virus, etc. F_1 = first fluid in a given culture. F_2 = fluid in a tissue culture after 1 change at 3 or 4 days until second change of fluid.

† Shows which fluid was used to infect each new tissue.

Culture age = time this combination of tissue, medium and virus was incubated before specimen was removed.

Tissue age = total time tissue has been in tissue culture.

Inoculum age = cumulative time virus has been in active tissue culture.

Calculated dilution of inoculum = dilution of the original inoculum that has occurred in the tissue culture at this stage.

‡ Number = day of death of mouse. S = mouse survived. No. in parentheses = death of mouse on day indicated is believed the result of causes other than MM infection.

K = mouse killed on day indicated with symptoms of MM infection.

Since tissue-to-fluid ratio was 1:100, virus content of fluid diluted 10^{-1} equals 10^{-3} in terms of amount of virus per unit mass of tissue.

MM virus in human testicular tissue. Tissue for these experiments was obtained from orchidectomy specimens from patients 57 to 78 years old, all of whom had carcinoma of the prostate gland except 1 who had benign prostatic hypertrophy. Two of them had received estrogen therapy, 1 for a period of 6 months and the other for 5 years prior to operation. Virus in an amount sufficient to give a concentration of 10^{-5} was added to the first set of cultures. Tests at 1, 2, and 3 days showed little virus. At 3 days the fluid was changed. Two days later virus was present in nearly maximum concentrations. This high level of virus was maintained for 3 days. At this time fluid was again changed. Subsequent tests showed that on the 10th day considerable amounts of virus were still present. For the 2nd tissue of this series (T_2), a tissue culture that had been maintained 8 days without virus was employed. The virus increased in amount and reached its maximum titer 7 days later, which was the 15th day of culture for this tissue. Subsequently the virus disappeared.

In the 3rd tissue and the 4th tissue, maximum amounts of virus were present at 8 days (See Table I, T_3 F_2 and T_4 F_2). The calculated dilution of the original inoculum in the 3rd fluid of the 4th tissue was 10^{-13}. The 10^{-1} dilution of this fluid contained sufficient virus to kill all 3 mice injected. Therefore it was concluded that MM virus multiplied extensively in human testicular tissue *in vitro*.

To check the identity of the virus, material from the last passage was shown to be infective after ether treatment overnight at refrigerator temperature and to cause flaccid paralysis of extremities of hamsters injected intramuscularly. One guinea pig injected intraperitoneally with 0.25 ml of the etherized fluid remained unaffected. Bacteriological tests of the fluid were negative.

Discussion. It is evident that the virus

multiplied extensively in tissue cultures of mouse testes and human testes. It is not clear whether the virus multiplied in the minute amounts of muscle from vessel walls, etc., or in some other tissue. Sections indicate that some cultures contained a small bit of striated muscle. In view of the relatively high titers of MM virus found in infected muscle-containing tissues in previously reported experiments(2) it seems quite possible that very small amounts of this type of tissue might give rise to easily detectable amounts of virus in tissue cultures.

The differences in rate of growth and period of survival of MM virus in cultures of human and mouse tissues may have clinical significance. If the slow rate of growth in human tissues *in vitro* is representative of the rate of growth *in vivo*, there should be more time for protective antibodies to be formed in infected persons than in mice or hamsters. The rapid decline in amount of virus in tissue cultures of mouse tissues may result from rapid killing of all susceptible cells, a factor that might contribute to the known severity of an infection in animals of that species.

Conclusions. MM virus multiplied extensively in 9 serial passages in mouse testicular tissue *in vitro* and in 4 serial passages in human testicular tissue *in vitro*. Fluid from the last tissue of each series represented a 10^{-13} dilution of original inoculum. These experiments do not exclude the possibility that the virus may have multiplied in the very minute amounts of muscle tissue present in the cultures. The virus multiplied more slowly and persisted longer in human tissue than in mouse tissue.

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Comparison of Excretion of Bromsulphthalein and Sodium Cholate after Intravenous Injection, Separately and Combined. (18440)

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(Introduced by W. J. Darby)

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Recently, interest has arisen in comparative studies of liver function tests, and the concept of association rather than dissociation of these tests has been brought to light(1,2). Although the bile salt tolerance test has been investigated intensively(3-9), it has not been subjected to a comparison with the bromsulphthalein test. The administration of bile salts has been observed to cause a retention of bromsulphthalein(10-12), but the blood

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