A MURINE VIRUS (JHM) CAUSING DISSEMINATED ENCEPHALOMYELITIS WITH EXTENSIVE DESTRUCTION OF MYELIN

I. Isolation and Biological Properties of the Virus*

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(Received for publication, May 16, 1949)

Several viral agents are known to cause spontaneous infection of the central nervous system of mice. Of these murine neurotropic viruses the so called Theiler's group (*e.g.* TO, FA, GD-VII), causing spontaneous mouse encephalomyelitis (1), has been most extensively studied since the paralytic disease they cause resembles the general picture and the pathological and epidemiological features of human poliomyelitis. Other agents with similar effects have been isolated as well (2). The reason for reporting another virus causing spontaneous encephalomyelitis in mice lies in the peculiar and distinctive pathological changes which it produces. While purely inflammatory lesions of both cord and brain are found, widespread demyelination of nerve tracts is the most prominent feature. This demyelinating process is sharply limited to the central nervous system; peripheral nerves are characteristically spared. In addition areas of focal necrosis are frequently found in the liver. This paper deals with the original isolation of the virus and with a description of its biological properties insofar as they have been determined. In the second paper, the characteristic pathological lesions are described, and the possible relationship of this disease to other demyelinating processes naturally or artificially produced in man or other animals is discussed.[1]

Isolation of the Virus

In the course of experiments on the epidemiology of diarrheal disease of suckling mice (4), two mice with spontaneous flaccid paralysis of the hind legs were discovered in the stock colony of the laboratory on August 14, 1947. These mice, 17 to 18 days old, were of the so called Schwentker strain of Swiss white mice. The line had been

* Aided by funds from the Grant Foundation.

Aided by a grant from The National Foundation for Infantile Paralysis.

[1] Brief reference to this virus with an illustration of the spinal cord lesions was included in a paper by Rustigian and Pappenheimer (3).

introduced into the laboratory a year previously by means of stock obtained from Mr. Victor Schwentker of Tumblebrook Farm, Brant Lake, New York. The experiment in which they were involved consisted of comparing the incidence of spontaneous diarrhea in four strains of mice kept under identical conditions throughout the course of a year (5). The two mice in question had shown no signs of diarrheal disease, and except for the obvious paralysis, showed no sign of illness.

These mice were sacrificed on the evening of the same day and a 20 per cent suspension in infusion broth of the two brains was inoculated intracerebrally into four 22 day old mice. On the 8th day one developed ruffled fur, and paralysis of the lower extremities. This animal was sacrified on the same day; the other three failed to show recognizable signs of disease during a period of 3 weeks. A 10 per cent suspension of brain from the sacrificed mouse was passed intracerebrally to eight mice. By the 5th day all mice were hunched, ruffled, and quiet; and the four sickest were sacrificed. Of the remaining four animals, two developed paralysis of the hind legs on the 9th day and died 24 hours later. The other two showed no further sign of the disease and seemed to have recovered completely at the end of 21 days. Consecutive brain passages have been carried on without difficulty since that time.

Repeated attempts to isolate a bacterial agent from infected material have met with consistent failure. The media inoculated as routine were blood agar plates, tryptic digest broth, and thioglycollate broth; occasionally cooked meat media and blood agar slants under anaerobic conditions were employed in addition. Attempts to isolate pleuropneumonia-like organisms by means of the technique described by Dienes (6) were unsuccessful.

With one possible exception no other instances of spontaneous infection have been recognized in our mouse colony as yet. In February, 1949, 18 months after the original isolation of the agent, a 3 to 4 week old mouse in the colony was was noted to exhibit spontaneous convulsions. The animal was sacrificed and a 10 per cent suspension of brain tissue was injected into six mice. Four of these became hyperexcitable at the end of a week. Pathological examination of their spinal cords showed lesions resembling those seen after injection of the virus previously isolated. Attempts to establish a virus by further passage have as yet met with failure.

The Disease Picture

The incubation period during the first five passages varied between 4 and 8 days after intracerebral inoculation. During the course of later passages the incubation period gradually shortened until it became stabilized at about the 37th passage. Mice usually showed signs of illness at the end of 24 hours and frequently died within 36 hours after receiving an intracerebral inoculation of a 10^{-1} dilution of infected brain substance. If a dilution of 10^{-3} was used for the inoculation, the incubation period was usually 24 to 48 hours longer.

The virus was first titrated using 15th passage material and an LD_{50} end point of $10^{-3.6}$ obtained. Titration of 38th passage material yielded comparable re-

sults. The titer of 44th passage material was $10^{-5.5}$—an increase of nearly 2 logs. Titrations of later passage material have with but few exceptions given LD_{50} end points in the range of $10^{-4.5}$–$10^{-5.5}$.

The first signs of illness have been ruffled fur, hunching, and general lassitude. In the earlier passages paralysis, usually limited to the hind extremities, followed. In later passages encephalitic signs have predominated and frank paralysis has rarely been observed before death ensued. Convulsions have been rare and hyperirritability has not been a prominent feature. Conjunctivitis has been an occasional finding, as have persistent rotary movements of the mouse in one direction or the other. An early manifestation of central nervous system involvement has been the animal's loss of ability to regain its feet after it has been rolled over onto its back. This clumsiness has appeared to be related to incoordination and weakness of the hind extremities. Tremors have been noted but infrequently.

The change in the picture from a predominantly paralytic to an encephalitic one coupled with a shortening of the incubation period raised the possibility that in the course of our mouse passages we had picked up another latent murine encephalomyelitic agent. Nine months after the original isolation, first passage brain material was removed from the dry ice cabinet, thawed, and injected into 3 week old mice. No recognizable disease resulted, and in spite of several blind passages no virus was isolated. When second passage material (which also had been frozen for 9 months) was thawed and tested in similar fashion, the virus agent was isolated without difficulty and carried through 15 successive intracerebral passages. During this time the change from a primarily paralytic to a primarily encephalitic picture correlated with a diminishing incubation period was noted. The lesions observed in the central nervous system and liver of these mice were quite similar to those observed in mice inoculated with the original line of virus. It seemed unlikely to us, therefore, that we had encountered another neurotropic agent during the course of intracerebral passages.

Following inoculation by other routes the symptoms have been much the same, although the incubation period has been somewhat longer and paralysis of the hind extremities has been noted more frequently.

Routes of Inoculation

Intracerebral inoculation has given the most satisfactory and consistent results. Mice of varying ages have appeared to be susceptible, although the highest concentration of virus has been found in the brains of 3 week old mice. The highest titer observed in 22 day old mice was $10^{-5.5}$ (44th passage material) and in 14 day mice, $10^{-4.8}$ (46th passage material). The effect of age on the susceptibility of the mouse was investigated in one experiment in which a virus pool was titrated simultaneously in mice of 3, 5, and 7 weeks of age. Although the final end points were not significantly different, the younger mice inoculated with any given virus dilution tended to succumb earlier as may be seen from Table I.

TABLE I

Titration of 50th Passage in Mice of Different Ages

Virus dilutions	10^{-1}			10^{-2}			10^{-3}			10^{-4}			10^{-5}			10^{-6}			LD_{50}		
Age of mice, *wks*	*3*	*5*	*7*	*3*	*5*	*7*	*3*	*5*	*7*	*3*	*5*	*7*	*3*	*5*	*7*	*3*	*5*	*7*	*3*	*5*	*7*
Day of death after inoculation*																					
2	6	2	1	2															$10^{-3.8}$	$10^{-3.5}$	$10^{-3.7}$
3			1	3			3														
4		2		2			1														
5			1		1	1	2						1								
6‡																					
7		3	1		6	4		5	6	1											
8			1			2			1		1	1			1						
9									1												
10+										1	1										
Survivors	0	0	0	0	0	0	0	2	0	5	5	5	6	6	5	7	7	7			
Totals	6/6	7/7	5/5	7/7	7/7	7/7	6/6	5/7	8/8	2/7	2/7	1/6	1/7	0/6	1/6	0/7	0/7	0/7			

* Deaths in 24 hours discounted as traumatic.

‡ Mice not observed on 6th day.

Of some interest was the finding that mice 6 to 7 days old were quite susceptible to the action of the virus, even though myelination of the brain and cord is not yet noticeable at this age. Typical signs developed within 24 hours of the intracerebral inoculation of 0.01 ml. of a 10 per cent suspension of the virus, and by the end of 48 hours

TABLE II

Results of Intramuscular Inoculation of 22 Day Old Mice

Passage No.	Dilution	Amount injected	Result
		ml.	
3	10^{-1}	0.1	0/7*
9	10^{-1}	0.2	0/9
20	10^{-1}	0.2	0/17
37	10^{-1}	0.2	2/7
38	10^{-1}	0.2	4/17
44 ‡	10^{-1}	0.2	5/10
	10^{-2}	0.2	5/10
	10^{-3}	0.2	1/10
	10^{-4}	0.2	1/10
49	10^{-2}	0.1	1/10

* Numerator, number of deaths; denominator, number of mice injected.

‡ LD_{50}, $10^{-1.7}$.

TABLE III

Titration of Virus by Intramuscular and Intracerebral Routes

Passage No.	Age of mice	Route of inoculation	LD_{50}
	days		
44	22	Intramuscular	$10^{-1.7}$
44	22	Intracerebral	$10^{-5.5}$
46	14	Intramuscular	$10^{-1.9}$
46	14	Intracerebral	$10^{-4.3}$
46	24	Intramuscular	$<10^{-1}$
46	24	Intracerebral	$10^{-4.4}$

death almost invariably ensued. The lesions which were characterized by an intense inflammatory reaction are described in the subsequent paper.

The *intraperitoneal* inoculation of the mice was followed quite consistently by the development of characteristic signs and finally death, although 3 week old mice were not so susceptible when infected by this route as they were by the intracerebral one. For example the LD_{50} of the 44th passage material when tested intracerebrally in 3 week old mice was $10^{-5.5}$; when tested intraperitoneally in mice of the same age the titer was $10^{-3.5}$. Two week old mice proved to be slightly more susceptible to the intraperitoneal inoculation of the virus and the difference of titer obtained by the two routes

of inoculation was not significant (46th passage tested in 14 day old mice intracerebrally, LD_{50}:$10^{-4.8}$; intraperitoneally LD_{50}:$10^{-4.5}$).

Mice 22 days old were inoculated *subcutaneously* with 0.2 ml. each of the 44th passage material in varying dilutions. After an incubation period of 6 days, signs of disease were observed and the first death occurred on the same day. The signs were predominantly encephalitic, although an occasional mouse showed paralysis of the lower extremities. This passage material gave an LD_{50} of $10^{-2.2}$ by this route as compared to $10^{-3.5}$ by intraperitoneal inoculation and $10^{-5.5}$ by the intracerebral route in mice of the same age.

Attempts to infect 3 week old mice by the *oral* and *intracutaneous* routes met with failure.

TABLE IV

Multiplication of Virus in Muscle following Intramuscular Inoculation

Time of sacrifice after inoculation	No. of mice sacrificed	Virus demonstrable	Titer (LD_{50})
15 min.	3	+	$<10^{-1}$
48 hrs.	3	+	$10^{-2.9}$
96 hrs.	3	+	$10^{-2.4}$
144 hrs.	3	+	$<10^{-1}$
Controls* D9, D10, S, S, S, S, S			

* D9, died on 9th day after inoculation. S, survived.

The *intranasal* inoculation of 10 per cent infected brain suspension brought down eight of eight mice within 3 days with typical signs of encephalitic involvement. The virus was isolated from the brains and cords of mice sacrificed when moribund.

The *intramuscular* inoculation of virus suspensions was followed only rarely by the development of disease. Early passage material yielded negative results in 3 week old mice. Thirty-seventh and later passage material gave occasional takes as shown in Table II. Fourteen day old mice proved to be more susceptible, but even with these younger animals the LD_{50} was $10^{-1.9}$, as compared with an LD_{50} of $10^{-4.8}$ when the same material was titrated intracerebrally in mice of a comparable age. The results of the various titrations are summarized in Table III.

Little evidence of myositis was found after intramuscular injection of the virus regardless of the age of the mice or of the death or survival of the animal. This stood out in contrast to the results obtained by Rustigian and Pappenheimer (3) working with the Columbia SK virus and the GD-VII and FA viruses. In spite of the paucity of pathological changes, however, evidence was obtained that actual multiplication of the virus did take place in the injected muscle.

Nineteen 3 week old mice were inoculated with 0.2 ml. of a 0.25×10^{-1} dilution of 38th passage material (about 500 intracerebral LD_{50}) in the thigh muscles of the right hind leg. At the end of 15 minutes, three mice were sacrificed, the injected muscles pooled, weighed, and ground with alundum and broth to make a 10 per cent suspension. After light cen-

trifugation the supernatant was removed, and tenfold dilutions made with distilled water. Each of the appropriate dilutions was injected intracerebrally into five or six 22 day old mice in 0.03 ml. amounts. Three more were sacrificed at 48 hours and the viral content of the pooled muscles titrated in the same manner. Similar titrations were carried out on mice sacrificed at 96 and 144 hours. The remaining inoculated mice, seven in number, were left as controls. The results are given in Table IV.

It can be seen that virus multiplication definitely took place, the highest titer being demonstrated 48 hours after inoculation. Two days later the titer was slightly lower, while 6 days after inoculation the virus content of the muscle had fallen to a titer of less than 10^{-1}. Of the control mice two died with definite signs of central nervous system involvement on the 9th and 10th days respectively; the other five remained well.

The *intravenous* inoculation of 3 to 4 week old mice with amounts of virus varying from 1,000 to 100,000 LD_{50} (as titrated by the intracerebral route) gave rise to obvious evidence of central nervous system involvement in sixteen of twenty-nine animals tested. The incubation period varied from 5 to 16 days, and once signs had developed the disease pursued its characteristic course to a fatal conclusion. The majority of these animals were sacrificed when moribund in order to secure material for pathological examination. One additional mouse sacrificed on the 3rd day after inoculation showed liver necrosis, although at this time no signs of illness had developed. There was no obvious correlation between the amount of virus injected and the resulting signs of disease and pathological picture.

Detection of Virus in Various Tissues

The JHM virus can be detected quite regularly in both brain and cord following intracerebral inoculation. Parallel titrations carried out on brain and cord material taken from the same animal showed approximately the same titer of virus in each.

The virus can be isolated from the liver as early as 2 days after intracerebral inoculation of the agent, and it appeared to be present in detectable amounts quite consistently at the time of death of the animal. This is in keeping with the frequent finding of areas of focal necrosis in this organ which were usually recognizable macroscopically. Virus isolated from the liver gave rise to usual signs of illness upon intracerebral inoculation of normal mice and the pathological findings were typical of the disease.

Virus has been isolated from the spleens, lungs, and kidneys of mice sacrificed when moribund after intracerebral inoculation. Virus isolated from these organs and injected into susceptible animals by the intracerebral route caused the development of typical disease; autopsy revealed the usual pathological lesions of the central nervous system.

We have been unable as yet to isolate virus from the blood of infected animals although samples taken at various stages of the disease have been tested for the presence of the agent. Since the most probable route travelled by the virus in its spread from brain to viscera is *via* the blood stream, our failure to demon-

strate a viremia is noteworthy. Seitz filtrates of ground-up intestine and intestinal contents, and similar material treated with streptomycin and penicillin failed to show the presence of virus.

Host Range

Attempts have been made to propagate the virus in other species and experiments employing young cotton rats, young Hisaw rats, and young hamsters gave positive results.

Two young cotton rats were injected intracerebrally with 0.06 ml. of a 10 per cent mouse brain suspension (50th passage material). Three days later one was found dead in its cage. The survivor appeared sluggish with ruffled fur; no definite paralysis was noted. This animal was sacrificed on the same day and a 10 per cent suspension of brain material inoculated into six mice by the intracerebral route. All appeared ill by the 3rd day; 24 hours later five were dead and the survivor was sacrificed in order to secure pathological material.

Eight Hisaw white rats, approximately 23 days of age, were inoculated intracerebrally with 0.07 ml. of a 10 per cent suspension of 50th passage material. On the 13th day after injection, one rat was discovered dead in its cage. Two others showed complete paralysis of the hind legs. Both animals were sacrificed and the brain of one was passed intracerebrally into white mice. These animals remained well, and in spite of two blind passages, no virus could be detected. A fourth rat was found dead on the 14th day. On the 21st day two animals showed incoordination. One of these rats was sacrificed on the next day and virus was demonstrated in its brain by mouse passage. On the 28th day one animal showed definite paralysis of the hind legs; it was sacrificed and attempts to isolate the virus from its brain were successful. Of the two survivors one remained well until it was sacrificed on the 70th day; the other showed incoordination over the course of a week but then recovered. Essentially similar results were obtained with another group of rats inoculated intracerebrally with the JHM virus for the purpose of securing pathological material.

Five 15 day old hamsters were inoculated intracerebrally with 0.05 ml. each of a 10 per cent suspension of 50th passage material. They remained well for 48 hours but were found dead on the morning of the 3rd day. From the pooled brains of two of the animals virus was isolated by the intracerebral inoculation of mice.

Three 25 day old hamsters were inoculated intracerebrally with 0.06 ml. of 50th passage 10 per cent brain suspension. At the end of 48 hours all three showed sluggishness and incoordinated movements with loss of righting reflexes. Two were sacrificed at this time and a 10 per cent suspension of ground-up brain passed by intracerebral inoculation into two more 25 day old hamsters as well as five mice. On the next day the third hamster of the original group suffered a series of convulsions and was sacrificed for pathological material. All the mice inoculated with the first hamster passage material developed signs of illness within 24 hours and were dead in 48 hours. Of the two second passage hamsters one showed gross incoordination by the 4th day at which time it was sacrificed; virus was isolated from its brain by the intracerebral inoculation of mice. The remaining hamster was found dead on the 5th day.

Four 3 to 4 week old guinea pigs remained well for 40 days after intracerebral inoculation of the virus. Eight additional 21 day guinea pigs were inoculated intracerebrally with the virus; all remained well for 21 days. Two animals sacrificed at this time showed but trivial lesions of the central nervous system. The other six showed no signs of illness during the ensuing 3 months of observation.

Two rabbits inoculated intracerebrally with 0.5 ml. of a 10 per cent suspension of infected mouse brain failed to develop signs of illness during a 3 months' period of observation. Experiments involving the inoculation of *Macaca mulatta* monkeys are now in progress.

Attempts to infect embryonated eggs of varying age have so far met with failure. The routes tried include the amniotic sac, the chorioallantoic membrane, the yolk sac, and the intracerebral methods of inoculation. Efforts to propagate the virus in tissue culture have not reached the point at which definite conclusions can be drawn.

Resistance to Physical and Chemical Agents

A number of physical and chemical agents were tested for their possible deleterious effect upon the JHM virus. Heating to 56°C. for 10 minutes inactivated the virus as did overnight exposure to ether (in saturated solution) or to 0.25 per cent formalin. After exposure to ultraviolet light for 1 minute the virus was still infectious but not after exposure for 5 minutes. When permitted to stand at room temperature infectivity persisted for at least 72 hours but could not be demonstrated at the end of 120 hours. Infectivity persisted for at least 3 hours at 37°C., for at least 21 days at 4°C., and for at least 10 months when frozen and stored in the CO_2 cabinet. A 10 per cent suspension of brain material was still infectious for mice at the end of 4 hours' exposure to 1 per cent phenol at room temperature. Exposure to 50 per cent alcohol for 30 minutes resulted in inactivation of the virus.

Filtration Experiments

A number of attempts were made to infect mice by the injection of material passed through filters of various types. The results were irregular. Success was had with Berkefeld V and W, Seitz EK, Mandler (regular porosity), and sintered glass "UF" filtrates, but it was not possible to repeat these results at will. The disease produced by the infectious filtrates was typical in every respect except for a slightly lengthened incubation period, and virus was recovered from the brains and cords of moribund mice. It appeared certain that filters which held back ordinary bacteria would under proper conditions permit the passage of an appreciable amount of virus. Since even in mouse brain the virus was present only in a comparatively low titer, the importance of reducing surface absorption to a minimum during the process of filtration was obvious. We obtained better results when hormone broth (which presumably contains a surface-active substance) was substituted for isotonic phosphate buffer solution (pH 7.1) as a suspending medium.

In one negative experiment in which a Seitz filtrate failed to produce disease when inoculated into mice, these animals were subsequently challenged by the intracerebral inoculation of 50 LD_{50} of virus. Fourteen of the twenty-four pre-

viously injected mice survived while all the controls died, thus suggesting that the filtrate contained enough virus to confer some immunity, although not enough to produce obviously recognizable disease.

Neutralization Tests

Attempts to define the serological properties of the JHM virus have been handicapped by our inability to produce satisfactory antisera against this agent in species other than mice. Guinea pigs and rabbits were subjected to long courses of intraperitoneal, subcutaneous, and intramuscular injections of the virus, but in no case did their sera show a satisfactory titer of antibodies. Our inability to stimulate the production of antibodies in these species may be related to the relatively small amount of virus apparently present in infected mouse brain; the highest titer obtained has been in the neighborhood of 10^{-6} M.L.D. per gm. of infected tissue.

Since apparently specific antibodies were found in the sera of mice exposed either naturally or artificially to the virus as well as in the sera of those animals "hyperimmunized" by repeated injections, the results are given in greater detail.

As a routine procedure serial tenfold dilutions of virus were made in buffer solution, and 0.2 ml. of each dilution was added to 0.2 ml. of undiluted serum which had been inactivated just prior to the test by heating to 56°C. for 30 minutes. After thorough shaking the tubes were placed in the refrigerator for 16 to 18 hours at 4°C. Five to seven mice were used for testing each dilution for residual viral activity by the intracerebral route. The LD_{50} of each mixture was calculated according to the method of Reed and Muench (7), and the neutralization index outlined by Paul (8). In a few tests serial dilutions of serum were set up against constant amounts of virus; in these instances the period of incubation was 1 hour in a water bath at 37.5°C. In addition the effect of complement in enhancing the neutralizing properties of a serum was investigated in these experiments.

Adult mice of the Schwentker strain were given a series of injections of the JHM virus by various routes: intramuscular, subcutaneous, intraperitoneal, and finally intracerebral. Approximately half of the mice survived the final intracerebral challenge inoculation and were bled 2 to 3 weeks after the last injection. Such sera when pooled showed a neutralizing index of over 1,000 when compared to normal rabbit serum.

When pooled sera from normal uninoculated Schwentker mice of a comparable age from our colony were tested, a titer of antibodies roughly equal to that shown by the immunized mice was found. Although these "normal" mice had shown no signs of spontaneous disease, they came from the same colony which had furnished the two original diseased mice; hence a possible explanation of the occurrence of these "natural" antibodies was that they resulted from the spread of latent infection with the JHM virus in the colony. That this explanation might be the correct one was supported by these findings:—

First: Pooled sera from 3 to 5 week old Rockland strain mice bled within 24 hours of their arrival in the laboratory, showed no significant antibody titer against the JHM virus.

Second: Two pools of sera from adult Schwentker strain mice of our colony bled shortly after the discovery of the JHM virus (in August, 1947) showed no antibody titer. In contrast, all adult mice from our colony bled 1 year or more after the isolation of the virus showed a significant level of antibody.

Third: Although 3 week old mice of the Schwentker strain had demonstrable circulating antibody, the titer was significantly lower than that observed in the pooled sera of adult mice of the same strain.

These data which are summarized in Table V suggested that the following course of events had occurred: Concomitant with or soon after the actual isolation of the virus from the mouse colony, there was a fairly wide spread of the

TABLE V

Summary of Neutralization Tests

Serum	Date bled	LD_{50}*	LD_{50} NRS‡ (control)	Log difference	Neutralizing index
Adult Schwentker mice (normal)	11–12–47	3.4	4.4	1.0	10
Adult Schwentker mice (diarrhea-recovered)	11–24–47	3.2	4.4	1.2	16
Adult Schwentker mice (Lansing-recovered)	8–18–48	1.2	4.4	3.2	1600
Adult Schwentker mice (normal)	12–11–48	<2.0	4.7	2.7+	>501
Adult Schwentker mice (normal)	1– 5–49	1.8	5.2	3.4	2520
3 week Schwentker mice (normal)	1– 5–49	3.3	5.2	1.9	80
Adult Rockland mice (normal)	1–20–49	4.6	5.0	0.4	<10
Adult Schwentker mice (recovered from JHM infection)	7–19–48	1.5	4.4	2.9	800
Adult Schwentker mice (hyper-immunized against JHM)	11–17–48	<1.0	4.7	3.7+	>5010

* Expressed as negative power of 10.

‡ Normal rabbit serum.

infection in a subthreshold form throughout the mouse colony. This infection did not cause solid immunity against the parenteral injection of the virus, but it did stimulate the production of circulating antibodies which could be recognized by neutralization tests.

Since several investigators (9–13) have presented evidence that the addition of fresh complement to the system enhanced the ability of an antiserum to exert a positive effect against the specific virus, the rôle of complement was investigated in connection with the neutralization of the JHM virus.

In these tests a constant amount of virus was tested against serial dilutions of the specific antiserum obtained from mice which had survived repeated injections of the JHM virus. Freshly thawed unheated antiserum, and antiserum which had been inactivated by heating to 56°C. for 30 minutes after thawing, were diluted in buffer solution, and 0.2 ml. of each dilution of unheated serum was placed in two tubes. To the first was added 0.2 ml. of fresh

undiluted guinea pig serum, and to the other the same guinea pig serum which had been inactivated by heating to 56°C. for 30 minutes. A similar test was set up in parallel employing the heated antiserum. To each tube was added 0.2 ml. of a suspension of virus so diluted that 0.03 ml. contained approximately 30 LD_{50}. Suitable complement controls were set up in the same manner. The tubes were shaken and permitted to stand in a water bath at 37.5°C. for 1 hour. At the end of this time 0.03 ml. of each serum-virus mixture was injected into each of five mice—each mouse thus receiving approximately 10 LD_{50} of virus. The results are given in Table VI.

These results suggested that the protective action of the anti-JHM serum was increased by the addition of complement in the form of fresh guinea pig serum.

TABLE VI

Effect of Complement upon the Ability of Specific Antiserum to Protect against JHM Virus (10 LD_{50})

Antiserum dilution (initial)	Antiserum (unheated) plus complement	Antiserum (unheated) plus inactivated complement	Antiserum (heated) plus complement	Antiserum (heated) plus inactivated complement
Undiluted	0/5*	0/5	0/5	0/5
1:10	0/5	0/5	0/5	1/5
1:100	1/5	5/5	0/5	5/5
1:1000	3/5	5/5	4/5	5/5

LD_{50}	Virus control (no serum or complement)	Complement control (no immune serum)
10	5/5	Unheated undiluted guinea pig serum 5/5
1	3/5	
0.1	1/5	Heated undiluted guinea pig serum 5/5

Except in the virus control titration each animal was injected with approximately 10 LD_{50} of virus.

* Numerator, number of deaths; denominator, number of mice inoculated.

This effect was obtained with freshly thawed unheated antiserum as well as with antiserum which had been heated to 56°C. for 30 minutes. The degree of enhancement was not marked, however, since it amounted to but slightly more than a tenfold increase.

Specific antisera against several neurotropic viruses have been tested against the JHM virus for possible protective effect. The sera employed were anti-SK (rabbit), anti-GD-VII (swine),[2] anti-pseudorabies (swine),[2] and anti-Mengo (swine).[2] In no instance was it possible to demonstrate a significant neutralizing effect. Pooled sera from mice hyperimmunized against the JHM virus failed to exert any protective effect against the Lansing virus.

[2] We are indebted to Dr. Hilary Koprowski of Lederle Laboratories, Inc., for making these sera available to us.

DISCUSSION

Since the pathological findings are described in a subsequent paper, it is obvious that an adequate discussion of our results cannot be given at this point. A few general remarks, however, about this agent may be appropriate here.

The causative agent of this demyelinating condition of mice appears to be a virus. Bacteriological cultures, both aerobic and anaerobic, have remained consistently negative. Neither bacteria nor rickettsia-like agents have been seen on direct smear or in tissue sections of infectious material. Attempts to isolate pleuropneumonia-like organisms have met with failure. On occasion the virus has passed through the usual bacterial filters. It is of interest that no inclusion bodies have been observed, and that so far we have not been successful in propagating this agent either in tissue culture or in the embryonated egg. Further attempts along these latter lines are now in progress.

Cotton rats, Hisaw rats, and hamsters appear to be susceptible to the intracerebral inoculation of this agent and the signs of disease and pathological picture produced in these animals are not incompatible with those seen in mice. Attempts to infect rabbits and guinea pigs have failed, while experiments employing *Macaca mulatta* monkeys are under way at this time.

As yet we have been unable to demonstrate a serological relationship with any of the comparatively few neurotropic viruses which we have tested. On the basis of the evidence produced thus far, the JHM virus does not appear to belong to the mouse encephalomyelitis (*i.e.* Theiler's virus) group since we have been unable to demonstrate any serological relationship nor have we succeeded in propagating it in the embryonated egg. In addition the failure of the JHM virus to cause myositis, and the distinctive hepatic and central nervous system lesions it produces lend further support to this view. It does not bear resemblance to the virus of lymphocytic choriomeningitis, another viral agent indigenous to mice, in that we have been unable to propagate it in the embryonated egg, and it does not appear to be pathogenic for guinea pigs. The possibility of herpes virus seems ruled out by our failure to find inclusion bodies or to propagate the agent in the chick embryo.

The virus can be propagated by serial passage in mice with ease. Evidence on this point regarding other rodents is as yet incomplete. Although the spontaneous disease has been observed but once in our mouse colony, there is serological evidence suggesting that infection with the virus may have become fairly common in subthreshold form at about the time the agent was first isolated The circumstances surrounding the introduction of the agent into our colony remain a subject for speculation.

SUMMARY

The isolation of a murine virus causing disseminated encephalomyelitis accompanied by extensive destruction of myelin in the central nervous system, and focal necrosis of the liver has been described.

Young mice can be infected by a number of parenteral routes. Both encephalitic and paralytic signs can be observed. After intracerebral inoculation the virus has been isolated from brain, spinal cord, liver, lung, spleen, and kidney, but not from blood or from intestinal walls and contents.

Hamsters, cotton rats, and Hisaw rats can be infected by the intracerebral route. Guinea pigs and rabbits appear to be insusceptible. Attempts to infect chick embryos have so far met with failure.

Under proper conditions the agent can pass through the usual bacterial filters. No inclusion bodies have been seen. No serological relationship to other neurotropic viruses has been demonstrated as yet.

We wish to thank Dr. T. H. Chu and Mr. L. J. Kunz for their valuable assistance in carrying out some of the experiments.

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