SPONTANEOUS ENCEPHALOMYELITIS OF MICE, A NEW VIRUS DISEASE

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Early in January, 1933, a young mouse with a flaccid paralysis of the hind legs was found among the stock mice of this laboratory. By the intracerebral inoculation of other mice with a suspension prepared from a portion of the brain and spinal cord of the paralyzed mouse, a similar condition was reproduced in normal animals. Histopathological studies of the brain of the original mouse showed a perivascular infiltration and scattered necrotic ganglion cells. In the spinal cord similar changes were observed, though to a much more marked degree. As mice are used extensively in the study of virus diseases, and spontaneous diseases of mice are of particular importance to workers in the virus field, it was decided that a thorough study of this disease might be of considerable value. A preliminary account of results obtained in the study of the virus which appears to be the etiological agent of this disease has already been published (1).

Incidence of the Disease

Since the discovery of the original paralyzed mouse, mice similarly affected have been found on numerous occasions among the stock mice. Five strains of the virus have been obtained from six mice found spontaneously infected. These five strains have all been propagated in series by the intracerebral inoculation of normal mice with a suspension of the brain and spinal cord from an infected mouse.

The majority of the spontaneously infected mice occurred in the Swiss strain purchased from several dealers. The infection, however, was not confined to this strain. No accurate statistics on the incidence of the natural occurrence of the disease are available, but in
general it appears to be very low, not more than one in about two thousand of purchased mice. Several mice became spontaneously affected in the laboratory during experiments with other infective agents. Mice found naturally infected have usually been young, most of them being approximately 6 or 7 weeks of age.

Strains of Virus Studied

Six attempts were made to transmit the infective agent from six spontaneously affected mice to normal animals. Five were successful, and the strains have all been maintained through serial mouse to mouse passages by means of intracerebral injection. All strains with the exception of strain IV have approximately the same degree of virulence. Strain IV is comparatively avirulent, and as a rule only a relatively few mice inoculated with it become paralyzed. With this strain it also sometimes occurred that none of the mice became paralyzed. The virus was maintained, however, by choosing one or two of the inoculated mice at random, removing their brains and cords, and injecting normal mice with a suspension of these organs. In certain experiments, on the other hand, it seemed almost as virulent as the other strains. By continuous passage in mice, all strains seemed to become more virulent. Cross immunity studies with four of these strains indicated that they are immunologically related. Immunological characteristics of the other two strains were not determined.

Symptomatology

The cardinal symptom observed in naturally infected mice was the paralysis of the hind legs, which was of a flaccid type. Apart from this the mice seemed well. In mice injected intracerebrally, after an incubation period ranging from 7 to over 30 days, the first sign was a weakness of one of the limbs, often a fore limb. This weakness progressed rapidly into a paralysis, often first of a fore limb and later of both hind limbs. At times, however, the initial paralysis was observed in the hind limbs. The extent of paralysis was usually much more marked in the hind than in the fore limbs, frequently progressing to the extent that the animal was entirely without the use of its hind legs. Progression in these cases was then only possible by the use of the fore limbs. In some mice

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1 One of the sick mice from which the strain known as strain II was procured, was kindly given to us by Dr. W. A. Sawyer.
the progress of the disease was arrested spontaneously at this stage, and the animal gradually recovered from the paralysis which in most instances leads to death. Complete recovery was seldom observed, however, and then only in animals which showed a mild degree of paralysis. Mice that lived after severe paralysis showed emaciation of the hind legs and other deformities of these extremities. Throughout the entire course of the disease, the mice appeared normal except for the obvious paralysis. In those markedly paralyzed in the hind quarters, a constant dribbling of the urine was often observed. The tail seemed never to become paralyzed.

**Influence of Age on Susceptibility**

The susceptibility of groups of mice of the same strain but of different ages to intracerebral inoculation of the virus was tested on numerous occasions. Invariably the younger mice were found to be more susceptible than the older. Almost all suckling mice died without any paralytic symptoms having been observed. With increasing age (Table I), the incidence of paralysis increases and the mortality decreases up to approximately 6 or 7 weeks of age. In mice older than this the incidence of paralysis again decreases and the incubation period becomes lengthened. In adult mice of various ages the difference in the morbidity rate is often insignificant, but any difference has always been in favor of the older mice being less susceptible. The difference between two groups of adult mice at times is shown only by the average incubation period being slightly longer in the older group. In adult mice the incubation period as
well as the morbidity rate gives much better indices of susceptibility than the time of death or the mortality rate. The age of the mice used in the majority of the experiments reported in this paper was from 6 to 10 weeks at the time of inoculation.

Intranasal Instillation of Virus

It was early found that mice could be infected by intranasal instillation of the virus. The number of mice which became paralyzed as a result of infection by this route was always much lower than the number becoming paralyzed after intracerebral inoculation with the same virus suspension. The percentage of mice developing paralytic symptoms following the intranasal instillation of virus varied considerably. In a large number of the experiments no mice became affected. The highest percentage of infection obtained by this route was 36. During the first twenty-one brain to brain passages, strain I produced the highest incidence of paralysis. In later passages, however, it very seldom produced paralysis when inoculated by the intranasal route. The comparatively avirulent strain IV, though injected by this route in several instances, produced paralysis in only one. The time of onset of symptoms following intranasal instillation of virus was always appreciably later than when mice were inoculated with the same material intracerebrally. Because of the low incidence of paralysis following intranasal instillation, experiments made to determine whether young mice were more susceptible than older ones when inoculated by this route were inconclusive.

Immunity Following Intranasal Instillation of Virus.—It was a matter of major interest to determine whether mice which had remained well after an intranasal instillation of virus developed immunity to a subsequent intracerebral injection.

Four groups of mice (Table II) were given intranasal instillation of virus—groups A and B with strain I, and groups C and D with strain II. Two additional groups, E and F, of the same age were included to serve as controls. 5 weeks afterwards, groups A, C, and E were inoculated intracerebrally with strain I, and groups B, D, and F with strain II. An additional group of mice, G, of the same strain but approximately 6 weeks younger, was inoculated intracerebrally with strain I.

As seen in Table II, the results indicate that previous intranasal instillation with strain I produced some degree of immunity to the
subsequent intracerebral injection of the same strain as well as to strain II. The immunity produced by intranasal instillation of strain II was comparatively weak to both strains, which in all probability is due to the fact that the suspension of strain I used for intranasal instillation was far more virulent or contained a higher concentration of virus than that of strain II. This presumption is substantiated by the fact that, following the intranasal instillation of virus, several mice which had received strain I became paralyzed, whereas all those that had received strain II remained well. The results of this experiment also indicate that there is some immunological relationship between the two strains. The influence of the age of the mice on their susceptibility is shown by the fact that all the mice in group G became paralyzed and died, whereas 50 per cent of those in group E survived.

Twelve additional experiments along the same lines have been performed, and the following observations have been made. Control mice injected intranasally with suspensions of normal mouse brains and spinal cords do not develop immunity. The degree of immunity following intranasal instillation of the virus seems to depend upon the virulence of the strain used. Strain I has produced a much higher degree of immunity than strain IV when administered intranasally. In fact, it was found very difficult to produce immunity with strain IV by this route, and in only one of three experiments did the results

<table>
<thead>
<tr>
<th>Mouse group</th>
<th>Strain of virus given intranasally</th>
<th>Strain of virus given intracerebrally 5 wks. later</th>
<th>No. of mice in group</th>
<th>Mice developing paralysis</th>
<th>Mortality</th>
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<tbody>
<tr>
<td>A</td>
<td>Strain I</td>
<td>Strain I</td>
<td>19</td>
<td>9</td>
<td>47.5</td>
</tr>
<tr>
<td>B</td>
<td>Strain I</td>
<td>Strain II</td>
<td>19</td>
<td>9</td>
<td>47.5</td>
</tr>
<tr>
<td>C</td>
<td>Strain II</td>
<td>Strain I</td>
<td>21</td>
<td>14</td>
<td>66.6</td>
</tr>
<tr>
<td>D</td>
<td>Strain II</td>
<td>Strain II</td>
<td>23</td>
<td>17</td>
<td>74</td>
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<tr>
<td>E</td>
<td>Normal control</td>
<td>Strain I</td>
<td>24</td>
<td>21</td>
<td>87.5</td>
</tr>
<tr>
<td>F</td>
<td>Normal control</td>
<td>Strain II</td>
<td>23</td>
<td>20</td>
<td>87</td>
</tr>
<tr>
<td>G</td>
<td>Normal controls 6 wks. younger</td>
<td>Strain I</td>
<td>22</td>
<td>22</td>
<td>100</td>
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</tbody>
</table>
SPONTANEOUS ENCEPHALOMYELITIS OF MICE

indicate that some immunity was produced with this strain. Total absence of immunity also was shown on one occasion in mice given strain I intranasally and tested afterwards with the same strain. From the results of nine experiments it was concluded that an intranasal instillation of the virus had produced a mild degree of immunity to a later intracerebral injection of the same or other strains. In this manner additional evidence was obtained to indicate that strains I, II, IV, and V were immunologically related.

As the control mice in these experiments were of the same strain and age, and were kept under the same conditions as the mice which received the intranasal instillation of the virus, it is apparent that the immunity in the latter group was greater than that which normally develops with age. In fact, in most of these experiments an additional group of control mice was used for the intracerebral immunity test. This control consisted of a group of mice, usually twenty-five in number, of the same strain but younger than those under experimentation. The results invariably showed that the degree of immunity was highest in the intranasally infected mice, lower in the normal controls of the same age, and lowest in the third group of normal younger mice of the same strain.

Whether the relative immunity which follows an intranasal instillation of the virus is accompanied by the development of neutralizing antibodies has not been determined. It must be emphasized that the degree of immunity produced by this means is rarely marked. As the test for the demonstration of neutralizing antibodies is still very much in the experimental stage, it did not seem at all promising to attempt to determine whether the sera of intranasally inoculated mice contained more neutralizing antibodies than the sera of normal controls of the same age.

Ultrafiltration Experiments with the Virus

Early in the study of this disease it was found that the virus passed readily through Seitz filters and all grades of Berkefeld filters. In these filtration experiments the virus suspension was always made in a diluent containing 10 per cent of normal monkey serum. Efforts were made to determine approximately the size of the virus particles by filtration through graded collodion membranes. A total of eleven experiments were made. The technique and the type of diluent were
essentially the same as used in the filtration of poliomyelitis virus (2). In Table III are summarized the results of the filtration experiments. The virus passed through a membrane with an average pore diameter of 60 mμ with relative ease, but as the pore size of the membranes was reduced less virus was demonstrated in the filtrate. On two occasions the virus was demonstrated after passage through a 43 mμ membrane but was never demonstrated in the filtrate of the 39 mμ membrane, which is therefore taken as the filtration end-point. Applying the formula of Elford to this ultrafiltration result, the particle size of the virus of encephalomyelitis of mice would appear to be 13 to 19 mμ, and it is therefore practically the same size as poliomyelitis virus (2, 3). The technical difficulties encountered in

<table>
<thead>
<tr>
<th>Average pore diameter of membranes mμ</th>
<th>No. of tests made</th>
<th>No. of times filtrate infective</th>
<th>Total No. of mice inoculated with filtrates</th>
<th>Paralysis in mice inoculated with filtrates</th>
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</thead>
<tbody>
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<td>2</td>
<td>2</td>
<td>22</td>
<td>18</td>
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<td>7</td>
<td>7</td>
<td>63</td>
<td>27</td>
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<td>17</td>
<td>10</td>
<td>184</td>
<td>35</td>
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<td>78</td>
<td>0</td>
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<tr>
<td>35</td>
<td>8</td>
<td>0</td>
<td>69</td>
<td>0</td>
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</table>

the filtration of poliomyelitis virus have been pointed out. The same difficulties were encountered with the virus of encephalomyelitis of mice. In the discussion of the filtration results with the virus of poliomyelitis, it was pointed out that the virus was in all probability smaller than calculated, and in fact this assumption was substantiated by Elford, Galloway, and Perdrau (3). The same criticism applies in the case of the virus of mouse encephalomyelitis, and it seems highly probable that the virus particles are considerably smaller than the ultrafiltration results indicate.

**Preservation of the Virus**

The preservation of the virus in infective brains and spinal cords in 50 per cent glycerine in the refrigerator was found to be a very
satisfactory method. Virus preserved in this manner proved to be infective for at least 150 days without apparent loss of activity. Its preservation by the method of desiccation in the frozen state, which has been so satisfactory for yellow fever virus, has not proven successful.

Distribution of the Virus in the Mouse

The infectivity of the blood of paralyzed mice was tested on several occasions. No virus was ever demonstrated. In addition, the infectivity of the blood was tested on the 1st, 3rd, 6th, and 8th days after an intracerebral injection of virus. On no occasion was there virus present. Following an intracerebral injection of virus, the brain was infective immediately, and the spinal cord usually became infective within 24 hours. In one experiment the spinal cord was tested for the presence of virus in one mouse on the 3rd day and in another on the 8th after intracerebral inoculation, with a negative result. In paralyzed mice the spinal cord was always infective and contained the virus in greater concentration than the brain. The amount of virus present was never very great, as titrations showed that 1 in 1000 was the highest dilution capable of producing infection.

The spleen, kidney, adrenal, and liver proved to be non-infective on two occasions, once in a mouse in the early stages of paralysis, and once in one which had been paralyzed for 20 days. The sciatic nerve on one occasion proved to be infective; the infectivity of other organs has not been tested.

Persistence of the Virus in Mice

Several experiments were undertaken to determine how long the virus persisted in the central nervous system of mice following intracerebral injection of the virus. For this purpose the mice were divided into two series, one consisting of mice which had become paralyzed, and the other of those which had remained well after an intracerebral injection of the virus. At irregular intervals one mouse of each group was killed and the infectivity of the brain and spinal cord was tested by the intracerebral injection of an emulsion of each organ into a group of mice. The results with the paralyzed mice
were briefly as follows: The virus was demonstrated in every instance, with one exception, up to 1 year after inoculation, which was the longest period tested. Judging by the average incubation period as well as by the number of mice becoming infected, there was invariably more virus in the spinal cord than in the brain. On several occasions the brain proved to be non-infective though the spinal cord contained virus. In general, there seemed to be a steady but slow decrease in virus content in both the brain and the spinal cord with the passing of time, although this decrease was more marked in the former organ.

The experiments to determine the persistence of the virus in mice which had remained well were performed with the comparatively avirulent strain IV. Approximately 100 mice were inoculated intracerebrally. All the mice which became paralyzed were removed and those which remained well were segregated and kept under observation. At irregular intervals up to 62 days after inoculation the infectivity of the brain and spinal cord was tested as has been described above. Virus was demonstrated on five occasions. The longest time that virus persisted in the central nervous system in this experiment was 48 days. In later experiments, however, virus was shown to persist for as long as 163 days following injection.

Neutralizing Antibodies

Preliminary investigations showed that the serum of mice which had been paralyzed for some time and were consequently immune to an intracerebral injection of virus, had developed a moderate capacity to neutralize the virus. The technique of the test which was finally adopted is briefly as follows:

The serum to be tested was mixed with an equal volume of three or more dilutions of filtered virus. These mixtures, with a similar set containing normal serum, were incubated for 3 hours at 37.5°C., and each was then injected intracerebrally into a separate group of twelve mice. Incubation of the mixtures was considered essential, as no protective action of the immune serum could be demonstrated if the inoculations were made shortly after mixing. In one experiment the mixtures were incubated for 24 hours. The results, however, were not satisfactory as a considerable proportion of virus had become inactive. Mice inoculated with the mixtures were kept under observation for at least 30 days.
SPONTANEOUS ENCEPHALOMYELITIS OF MICE

The amount of neutralization obtained was not great, but a difference between the serum from the immune mice and that from the normal mice was invariably manifest.

An attempt was made to determine whether the resistance to infection developing with age in mice was accompanied by development of neutralizing antibodies.

The mice were divided into two large groups of equal numbers. One group was kept as the control, and the other was injected intracerebrally on Mar. 12 with strain IV of the virus. Nearly all the inoculated mice became paralyzed. On Apr. 12 a number of the paralyzed mice were bled, and their sera were pooled and designated as serum A. On the same date a similar serum pool was prepared from the normal controls and called serum B. These two serum pools were sealed in test tubes and stored in the ice box. On July 12 two other pools of sera were prepared, one, serum C, from paralytic mice, and the other, serum D, from the controls. Neutralization tests were done with all four of the serum pools in one experiment. Three dilutions, 1 in 10, 1 in 100, and 1 in 1000, of the virus (strain I) were used. Equal amounts (0.5 cc.) of each serum were mixed with each dilution of the virus. The mixtures were incubated for 3 hours at 37.5°C., and twelve mice were inoculated intracerebrally with each mixture. The mice were kept under observation for 40 days. The results obtained are summarized in Table IV. The mice which died before the 7th day were excluded.

An analysis of the results shows that there was a significant difference between the normal and immune sera of the first pools (sera A and B) in their power to retard the onset of paralysis. This difference was less significant between the immune and the normal sera.

### TABLE IV

<table>
<thead>
<tr>
<th>Serum pool</th>
<th>Date when obtained</th>
<th>Mice source of serum</th>
<th>No. of mice used in test</th>
<th>Percentage of mice paralyzed</th>
<th>Average time interval between injection and onset of paralysis</th>
<th>Percentage of mice which died</th>
<th>Average time interval between injection and death</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>Apr. 12</td>
<td>Paralyzed</td>
<td>35</td>
<td>80</td>
<td>16.7</td>
<td>83</td>
<td>19.7</td>
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<tr>
<td>B</td>
<td>Apr. 12</td>
<td>Normal</td>
<td>31</td>
<td>84</td>
<td>12.7</td>
<td>74</td>
<td>16.3</td>
</tr>
<tr>
<td>C</td>
<td>July 12</td>
<td>Paralyzed</td>
<td>33</td>
<td>55</td>
<td>15.2</td>
<td>67</td>
<td>18.2</td>
</tr>
<tr>
<td>D</td>
<td>July 12</td>
<td>Normal</td>
<td>31</td>
<td>68</td>
<td>13.2</td>
<td>61</td>
<td>17.0</td>
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</table>
of the second pools (sera C and D). With regard to the incidence of paralysis, the only significant difference between the sera taken after 1 month and the sera taken after 4 months was that the second immune pool (serum C) prevented paralysis in a greater number of mice than did the first immune pool, and the difference between the two normal pools in this respect was less evident. The mortality rate showed no significant difference, although there was a suggestion of a lower mortality with serum C than with serum A.

This experiment consequently does not settle the question of whether the relative immunity of older mice is accompanied by a development of neutralizing antibodies. The results show, however, that neutralizing antibodies are produced as a consequence of infection with the virus, and that for some time, at least, there is an increase of the antibodies manifested by the fact that the serum obtained 4 months after infection was more potent than that obtained 1 month after infection.

Relation of the Virus of Mouse Encephalomyelitis to the Virus of Human Poliomyelitis

Because of several points of similarity between the virus of poliomyelitis and the virus under study, several experiments were performed to determine whether there was a relationship between them. This became especially important as two of the strains of virus were obtained from mice which had been inoculated with the virus of human poliomyelitis. These two strains are referred to in this paper as strains II and IV.

Rhesus monkeys were inoculated intracerebrally or intracutaneously with suspensions of mouse brain containing the virus of mouse encephalomyelitis. In every instance the inoculated monkeys remained well, and when given an intracerebral injection of poliomyelitis virus later all proved to be susceptible. In all, eleven rhesus monkeys were inoculated with strains I and II. In addition three African green monkeys were inoculated, two with strain I and one with strain II; these also remained well.

Several neutralization tests were performed with the virus of encephalomyelitis and poliomyelitis immune monkey sera. For controls, serum obtained from two monkeys before they were infected with the virus of poliomyelitis was available. The results of these tests showed that there was no significant difference in the neutralizing action of the serum of a normal monkey and that of the same animal after it had become immune to the virus of poliomyelitis.
As mentioned above, it has been shown that mice could be rendered relatively immune to encephalomyelitis as a result of an intranasal instillation of the encephalomyelitis virus. It was considered of interest to determine whether mice could be immunized against encephalomyelitis by the intranasal instillation of poliomyelitis virus.

In the first experiment, twenty-five mice were given four intranasal instillations of poliomyelitis virus. One month after the last inoculation, these mice, as well as thirty normal mice of the same strain and age, were given an intracerebral injection of the virus of encephalomyelitis. As the results seemed to indicate that the previous instillation of poliomyelitis virus had conferred a slight degree of immunity to a subsequent injection of the virus of encephalomyelitis, the experiment was repeated on a larger scale. The results of the second experiment showed no significant difference between the immunized mice and the normal controls.

The results of all these experiments suggest that there is probably no relationship between the virus of poliomyelitis and the virus of mouse encephalomyelitis.

Immunity

Paralyzed mice, as far as could be determined, appeared to be solidly immune to a second intracerebral injection of virus. Virus was still present in the central nervous system at this time, as a rule. Whether this immunity is permanent and persists after the disappearance of virus from the paralyzed mice has not been determined. A large proportion of the mice which remained well after an intracerebral injection of the virus were immune to a subsequent intracerebral injection of the virus.

The degree of immunity produced by age or after intranasal instillation of the virus is in many instances insignificant and never approaches that produced by intracerebral inoculation of the virus. Mice inoculated intraperitoneally with a massive dose of the virus did not develop immunity to a subsequent intracerebral injection. The application of virus to the scarified skin did not lead to immunity, nor could immunity be demonstrated in normal mice placed in contact with mice infected either spontaneously or artificially by intracerebral or intranasal inoculation of virus. In these experiments, it must be emphasized that the control mice were of the same strain and
age and were kept under the same conditions as the experimental mice. The degree of immunity often produced by intranasal instillation of the virus was so slight that in order to obtain significant results large numbers of mice had to be used.

Epidemiology

The incidence of spontaneous paralysis is very low. There is apparently a natural immunity which develops with the increase of age. Following the intranasal instillation of virus, comparatively few mice develop paralysis. A relative immunity, however, is often produced. Furthermore, mice which had become spontaneously infected were almost invariably young. These facts would seem to indicate that the virus is widespread and that only unusually susceptible mice or possibly mice exposed to massive dosages of virus develop clinical signs of infection. Suckling mice under natural conditions might become infected and die without showing signs of paralysis. The majority of the mice appear to build up an immunity gradually. Contact experiments in which apparently normal mice were placed in the same cages with those infected either by intracerebral inoculation or by intranasal instillation have not produced evidence of infection as a result of the contact. Nor did these mice develop immunity as compared with the control mice which were kept in a similar environment but not in contact with infected animals. The addition of several mice found spontaneously infected to a cage of normal mice likewise failed to produce infection in the normal animals. In this experiment the mice were allowed to become overcrowded by breeding, and as more spontaneously infected mice became available, they also were added. The experiment was allowed to continue for several months. At its termination six mice were taken at random from this case and the infectivity of the spinal cords tested. No virus was demonstrated. The failure to produce infection or immunity by intraperitoneal injection of massive doses of virus, the absence of virus at any time in the blood, and the absolute neurotropism of the virus itself make it seem probable that blood-sucking insects are not the means of spreading the infection. Nevertheless, as our experimental mice at times were heavily infested with a species of mite, this arthro-
pod was tested for its capability of transmitting the infection, by placing normal mice in jars containing many mites obtained from infected mice. The results were entirely negative.

The presence of healthy carriers would help greatly to explain the ascertained facts. That they can be produced has been adequately demonstrated, but whether they are capable of giving off virus has not been determined.

**Pathology**

Macroscopically nothing abnormal has been observed. Microscopically there is a perivascular round celled infiltration throughout the central nervous system, which is particularly marked in the spinal cord. An acute necrosis of ganglion cells, especially marked in the anterior horn cells, is a striking feature. These necrotic changes have been observed before the onset of paralysis. The process is exceedingly acute, necrosis being soon followed by neuronophagia. The ganglion cells in other parts of the cord are not affected to the same extent as those of the anterior horn. The cells of the posterior root ganglia appear to remain unaffected.

Mice which have been paralyzed for several months show a relative decrease in the number of anterior horn cells. At this stage, virus can still be demonstrated in the spinal cord. Perivascular infiltration is present, although to a much less extent. No intranuclear inclusions have been observed in infected brains or spinal cords.

**SUMMARY**

1. The characteristics of a filterable virus obtained from mice found spontaneously paralyzed and showing lesions of encephalomyelitis are described.

2. The course of the disease in mice, following intracerebral inoculation, is briefly as follows: After an incubation period varying from 7 to over 30 days a flaccid paralysis of one of the limbs appears. This paralysis usually spreads rapidly until all four limbs are affected. Young mice are more susceptible than older ones, and very young mice, less than 4 weeks of age, usually die without showing signs of paralysis.

3. Adult mice often show no signs of infection after an intracerebral inoculation of virus. A number of these mice, although showing no signs of paralysis, nevertheless have become infected, a fact which is
demonstrated by recovery of the virus from the mice as well as by histopathological studies.

4. Intranasal instillation of the virus is the only other method of producing the infection. This method, however, produces paralysis in only a small percentage of the mice. Following intranasal instillation of the virus, there often develops a slight immunity to a subsequent intracerebral injection of virus.

5. The paralysis in the surviving mice recedes gradually, but a permanent residual paralysis, usually of the hind legs, is almost invariable. Such mice, however, are virus carriers, as the virus can be recovered from the spinal cord for 1 year after infection.

6. Paralyzed mice are immune to a subsequent intracerebral injection of the virus. There is evidence that neutralizing substances are present in the immune mice. A considerable proportion of the mice which have remained well after an intracerebral injection of virus are immune to a second injection.

7. The virus resists the action of 50 per cent glycerine at from 2–4°C. for at least 150 days. It passes all grades of Berkefeld filters with ease. By the use of graded collodion filters, the size of the virus particle has been determined to be probably about 13 to 19 μm.

8. The virus of mouse encephalomyelitis is not pathogenic for rhesus monkeys. No evidence of immunological relationship with the virus of human poliomyelitis has been obtained.

9. The anatomical basis of the paralysis is an acute necrosis of the ganglion cells of the anterior horn of the spinal cord. Isolated ganglion cells of the cerebrum also undergo necrosis. Following the acute necrosis of the ganglion cells, there is a marked neuronophagia. A perivascular infiltration is observed in the brain and spinal cord.

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