This study had its inception when a few preliminary experiments were being performed for the purpose of procuring certain conditions in mice infected intranasally with encephalomyocarditis virus. In accordance with our customary laboratory procedure, duplicate experiments were run with consideration for sex. It was found that under certain conditions male mice were more susceptible to infection with encephalomyocarditis virus than females. This was not too surprising since, as Rivers (1948) points out, numerous neurotropic viruses show a greater predilection for males. This is true, for example, for St. Louis encephalitis, Japanese B encephalitis, and Western equine encephalitis. Upon further investigation it was shown that the sex of the animal from whose brain tissue the virus inoculum was prepared, as well as the sex of the animal to be infected, is a significant fact in determining the severity of the infection. The work reported here is in support of this contention.

MATERIALS AND METHODS

The mice used in this study were a Swiss strain, weighing 18 to 22 grams, purchased from R. F. Beyer and Son, Billings, Missouri. All animals received tap water and Purina chow pellets, ad libitum, under both normal and experimental conditions.

The mice were always infected by intranasal instillation of 0.03 ml of the viral suspension while the animals were under light ether anesthesia. In all cases 30 mice comprised an experimental group.

Concentrated encephalomyocarditis viral suspensions were prepared by homogenizing the brains from recently dead infected animals or dying animals, both groups necessarily showing paralytic symptoms, with sterile 50 per cent glycerol in a sterile pestle and mortar. A proportion of one brain to 5 ml of 50 per cent glycerol was used. The concentrated homogenate was centrifuged for 20 minutes at 2,500 rpm and the supernatant aseptically transferred to a sterile vaccine bottle. Two suspensions of concentrated encephalomyocarditis virus were prepared in this manner, one derived exclusively from the brains of male mice, the other from female mouse brains. All the work reported here was done with these two suspensions or with dilutions thereof. Most of the experiments were run simultaneously; where this was not possible controls were run in order to determine that the original titer of the virus suspensions had not changed significantly. Thus, it is significant that any one of the experimental groups of mice may be compared to any other group in this report. Suspensions of male and female brain homogenates from normal, apparently healthy, mice were prepared in the same manner described for infected brains.

When not in use all brain homogenates were stored in the freezer compartment of a refrigerator. In order to facilitate presentation of the data, each experimental group of 30 mice is identified here by a code number.

RESULTS

Figure 1 records the mortality rates for groups of mice in which virus suspensions derived exclusively from male or female brains were used to infect both male and female groups of animals. The virus suspension used here was the concentrated mouse brain homogenate described in Materials and Methods (1 brain:5 ml 50 per cent glycerol). The data for male mice infected with virus suspension from male brains may not be compared to that for male animals infected with virus suspension from female brains because it is not known that the titers are comparable. Similarly, the groups of female animals infected with virus from male and those infected with
virus from female brains are not comparable. Because the virus titers from both the male and
female brain homogenates are high, there is almost 100 per cent mortality in all groups, and the mortality curves fall fairly close together. It is still obvious, however, that there is a significant difference in mortality rate between male (4-40 E) and female (4-40 F) mice infected with virus from female brains, the male mice showing more susceptibility. The difference between male and female mice infected with encephalomyocarditis virus from male brains is too small to be considered significant. In an attempt to magnify the sex difference shown in figure 1, the virus suspensions used in the 4-40 series of mice were diluted 1:1 with 50 per cent glycerol and the experiment repeated; the data are presented in figure 2.

It is clear at sight that there is a distinct difference in susceptibility to encephalomyocarditis infection between male and female mice and that this difference is expressed regardless of the sex of the animal from which the virus is harvested. This difference in susceptibility is manifest primarily as a difference in mortality rate and is greater during the earlier and middle periods of the experiment, approximately 6 to 10 days, after which time all the curves begin to coalesce. In both cases the male animals are more susceptible to infection than the female. For example, after 6 days the female mice infected with virus prepared from female brains had 23 survivors, but only 6 males similarly infected survived. Also after 6 days the female mice infected with virus from male brains had 26 survivors, but only 13 males similarly infected survived.

This experiment was repeated with the same virus suspensions used in the 4-51 series of mice (figure 2) but further diluted 1:1 with sterile 50 per cent glycerol. These data are recorded in figure 3. A further dilution of the encephalomyocarditis virus which was used in the 4-51 series served to obliterate the particular sex differences manifest with the more concentrated virus suspensions (compare figures 2 and 3). Although there is one highlighted difference at the seven day incubation period between male and female mice infected with female brain homogenate, this difference is fleeting.

The mortality curves for male and female mice infected with virus from female brains (curves 4-57E and 4-57 F in figure 3) show fewer surviving animals than do the curves for either male or female mice infected with virus from male brains (curves 4-57 C and 4-57 D in figure 3). It would appear either that the factor(s)

Figure 1. The mortality rate of mice infected with concentrated encephalomyocarditis viral suspensions derived from the brains of male or female mice.

Figure 2. This series of mice infected with the encephalomyocarditis viral suspensions used in the 4-40 series (figure 1) but diluted 1:1 with 50 per cent glycerol. Legend for curves same as in figure 1.
responsible for the greater susceptibility on the part of the male animals to encephalomyocarditis infection, whatever that factor may be, was diluted out or that some innate greater predisposition to encephalomyocarditis infection on the part of the males required a moderately high virus titer for its expression. What was suggested by the curves in figure 3 was that encephalomyocarditis virus derived from infected female brains was more virulent than that prepared from male brains. This postulate required further substantiation since results similar to those shown in figure 3 could be attributed to major differences

respectively). This experiment was performed and is illustrated graphically in figure 4.

Mixing of equal parts of female and male brain homogenate containing encephalomyocarditis virus markedly reduced the mortality rates for both male and female animals, and although the male mice still demonstrated a greater susceptibility to infection this difference was considerably less than would have been expected from the results shown in figure 2.

The curves of figure 4, compared to those in figure 2, indicate that a mixing of encephalomyocarditis virus homogenates from male and female

brains results in a neutralization of the virulence of the virus. Assuming the validity of the postulate suggested by the curves in figure 3 that encephalomyocarditis virus suspensions derived from female brains have a greater virulence than virus prepared from male brains, it would follow from the data illustrated in figure 4 that there is some factor present in male brains which neutralizes in part the virulence of the virus derived from infected female brains. If there were present in the male and female brains of mice some factor or factors which modify the virulence of encephalomyocarditis virus, it would be significant to determine whether such a factor is
present in the brains of uninfected mice as well as in the brains of infected mice. The following experiment was designed to resolve this question and to further substantiate the concept that a factor is present, presumably in male mice, which can antagonize the virulence of encephalomyocarditis virus.

Male or female mice were infected with the encephalomyocarditis viral preparations used in the 4-51 series (figure 2) to which was added an equal volume of concentrated normal male or female brain homogenate in 50 per cent glycerol, prepared as described in Materials and Methods. The variable factors of the sex of the mice to be injected, the sex of the animals from which the infected brain homogenates were prepared, and the sex of the mice from which the normal brain homogenates were prepared, when tabulated in all possible combinations, result in the series of eight experimental groups of animals shown in table 1.

It will be noticed that such preparations would yield virus concentrations similar to that used in the 4-57 series (figure 3), in which the factor of greater susceptibility of the male animals to encephalomyocarditis virus was markedly reduced, if not eliminated. Four control groups of mice, consisting of male and female mice inoculated with 0.03 ml of the concentrated suspensions prepared from normal male or female mouse brains, were run simultaneously with these.

The data for these experiments are recorded in figures 5, 6, and 7.

### TABLE 1

The inoculation of mice with encephalomyocarditis virus prepared from male or female brains and diluted with normal male or female brain homogenates. The combinations presented here serve as legend for data presented in figures 5 and 6.

<table>
<thead>
<tr>
<th>CODE NO. OF GROUP OF MICE</th>
<th>SEX OF INFECTED MOUSE</th>
<th>SEX OF MICE FROM WHOM ENCEPHALOMYOCARDITIS VIRUS IS PREPARED</th>
<th>SEX OF NORMAL MICE FROM WHOM BRAIN HOMOGENATES WERE PREPARED AND ADDED TO ENCEPHALOMYOCARDITIS SUSPENSIONS IN EQUAL PROPORTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-61 F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>4-61 H</td>
<td>F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>4-61 L</td>
<td>M</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>4-61 N</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>4-61 E</td>
<td>M</td>
<td>M</td>
<td>F</td>
</tr>
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<td>F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
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<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>4-61 M</td>
<td>F</td>
<td>M</td>
<td>M</td>
</tr>
</tbody>
</table>

Figure 5. The mortality rate of mice infected with encephalomyocarditis viral suspension prepared from male or female brains which is diluted with an equal part of normal male or female brain homogenate. See table 1 for details of individual curves.

Figure 6. Same legend as in figure 5.

If female brain tissue contains a factor which enhances the virulence of encephalomyocarditis virus, we would expect to find the following comparisons to be substantiated by our data:

1. Male mice infected with encephalomyo-
carditis virus from female brain homogenate diluted with normal female brain suspension (4-61 L, figure 5) should show an enhanced mortality rate as compared to male mice infected with encephalomyocarditis virus from female brain homogenate diluted with normal male brain suspension (4-61 F, figure 5).

(2) Similarly, female mice infected with encephalomyocarditis virus from female brain homogenate diluted with normal female brain suspension (4-61 N, figure 5) should show an enhanced mortality rate as compared to female mice infected with encephalomyocarditis virus from female brain homogenate diluted with normal male brain suspension (4-61 H, figure 5).

(3) Male mice infected with encephalomyocarditis virus from male brain homogenate diluted with normal female brain suspension (4-61 E, figure 6) should show an enhanced mortality rate as compared to male mice infected with virus from male brain homogenate diluted with normal male brain suspension (4-61 I, figure 6).

(4) Female mice infected with encephalomyocarditis virus from male brain homogenate diluted with normal female brain suspension (4-61 G, figure 6) should show an enhanced mortality rate as compared to female mice infected with virus from male brain homogenate diluted with normal male brain suspension (4-61 M, figure 6).

In every one of these four instances the expected findings are substantiated by the data shown in figures 5 and 6.

If male brain tissue contains a factor which reduces the virulence of encephalomyocarditis virus, we would expect the following to be substantiated by our data:

(5) Male mice infected with encephalomyocarditis virus from male brain homogenate diluted with an equal volume of 50 per cent glycerol (4-57 C, figure 3) should show an enhanced mortality curve when compared to male mice infected with the virus suspension from male brains diluted with an equal volume of homogenate of normal male brains in 50 per cent glycerol (4-61 I, figure 6).

(6) Similarly, male mice infected with encephalomyocarditis virus from female brain homogenate diluted with an equal volume of 50 per cent glycerol (4-57 E, figure 3) should show an enhanced mortality rate when compared to male mice infected with the virus suspension from female brains diluted with an equal volume of glycerol (4-57 F, figure 3) should show an enhanced mortality rate when compared to female mice infected with the virus suspension from female brains diluted with an equal volume of homogenate of normal male brains in 50 per cent glycerol (4-61 H, figure 5).

All the statements except (6) and (8) are substantiated by the data indicated above.

If there is a factor present in female brains which tends to enhance the virulence of the encephalomyocarditis virus, we should also expect to find that:

(9) Male mice infected with encephalomyocarditis virus from male brain homogenate diluted
with an equal volume of 50 per cent glycerol
(4-57 C, figure 3) should show a reduced mortality
rate when compared to male mice infected with
the virus suspension from male brains diluted
with an equal volume of homogenate of normal
female brains in 50 per cent glycerol (4-61 E,
figure 6).

(10) Male mice infected with encephalomyo-
carditis virus from female brain homogenate
diluted with an equal volume of 50 per cent glycerol
(4-57 E, figure 3) should show a reduced mortality
rate when compared to male mice infected with
the virus suspension from female brains diluted
with an equal volume of homogenate of normal female brains in 50 per cent glycerol (4-61 L, figure 5).

(11) Female mice infected with encephalomyo-
carditis virus from male brain homogenate diluted
with an equal volume of 50 per cent glycerol
(4-57 D, figure 3) should show a reduced mortality
rate when compared to female mice infected with
the virus suspension from female brains diluted
with an equal volume of homogenate of normal female brains in 50 per cent glycerol (4-61 G, figure 6).

(12) Female mice infected with encephalomyo-
carditis virus from female brain homogenate diluted
with an equal volume of 50 per cent glycerol
(4-57 F, figure 3) should show a reduced mortality rate when compared to female mice infected with the virus suspension from female brains diluted with an equal volume of homogenate of normal female brains in 50 per cent glycerol (4-61 N, figure 5).

In every case where these comparisons (9-12)
amare the expected results are substantiated
by the data in figures 3, 5, and 6.

If there is a factor present in male brains which
tends to reduce the virulence of the encephalo-
myocarditis virus, we should also expect to find
that:

(13) Female mice infected with encephalomyo-
carditis virus from male brain homogenate diluted
with an equal volume of 50 per cent glycerol
(4-57 D, figure 3) should show an enhanced mortality rate when compared to female mice infected with the virus suspension from male brains diluted with an equal volume of homogenate of normal male brains in 50 per cent glycerol (4-61 M, figure 6).

(14) Male mice infected with encephalomyo-
carditis virus from male brain homogenate diluted
with an equal volume of 50 per cent glycerol
(4-57 C, figure 3) should show an enhanced mortality rate when compared to male mice infected with the virus suspension from male brains diluted with an equal volume of homogenate of normal male brains in 50 per cent glycerol (4-61 I, figure 6).

(15) Male mice infected with encephalomyo-
carditis virus from female brain homogenate diluted
with an equal volume of 50 per cent glycerol
(4-57 E, figure 3) should show an enhanced mortality rate when compared to male mice infected with the virus suspension from male brains diluted with an equal volume of homogenate of normal male brains in 50 per cent glycerol (4-61 F, figure 5).

(16) Female mice infected with encephalomyo-
carditis virus from female brain homogenate diluted
with an equal volume of 50 per cent glycerol
(4-57 F, figure 3) should show an enhanced mortality rate when compared to female mice infected with the virus suspension from female brains diluted with an equal volume of homogenate of normal male brains in 50 per cent glycerol (4-61 H, figure 5).

For statements (13) and (14) these comparisons
are substantiated clearly by the data in figures 3,
5, and 6. For (15) the comparison also is sub-
stantiated but perhaps is not as conclusive as in
(13) and (14). Only in (16) is there a clear lack
of correlation of expectations.

The controls presented in figure 7 resulted in a
few deaths in the various groups of mice, but it is
doubtful that these few deaths played any sig-
nificant role in affecting the mortality curves in
figures 5 and 6. The mice in figures 5 and 6
received in their inoculum only one-half the
quantity of the normal brain homogenate as the
animals in figure 7. In addition, none of the
control mice showed any paralytic symptoms
either prior to or after death.

DISCUSSION

The data presented here lend themselves to
the following hypothesis. There is a factor(s)
present in the brains of female mice which
enhances the mortality rate of male and female
mice infected with encephalomyocarditis virus.
This factor is antagonized by some factor(s)
present in the brains of male mice. It is not known
whether the factor from either male or female
brains acts by modifying the virulence of the
virus or by affecting the resistance of the host. Both factors appear to be present in the brains of mice whether the animals are infected with encephalomyocarditis virus or are normal apparently healthy animals. If concentrated virus suspensions are used, the effects of the factors present in the brains of male or female mice are suppressed or overshadowed by a greater susceptibility to encephalomyocarditis infection manifest by male mice. With more dilute encephalomyocarditis viral suspensions this greater susceptibility to infection for male animals does not appear or its effect is obscured by the factors postulated to be present in male and female mouse brains.

Perhaps explanations other than the one proposed here may be presented which still will be in accord with these data. It is noteworthy, however, that for the 16 points of comparison that were made among the various groups of animals studied, 12 or possibly 13 were in support of the concept proposed here.

This author will not indulge in the luxury of speculating as to what the factors in the male and female brains might be that are responsible for the results presented here. It might be significant to mention that the brains, as isolated for this work, contained the entire brain and a small portion of the cord but were probably free of the pituitary. It is important to determine whether a phenomenon similar to that reported here can be found for other neurotropic viruses.

ACKNOWLEDGMENT

The author is indebted to Messrs. William Larsen and Charles L. Wittenberger for their very capable technical assistance.

SUMMARY

The infecting of male and female groups of mice with encephalomyocarditis viral suspensions prepared from brains of male and female animals and appropriately diluted with brain homogenates from normal male and female mice resulted in a series of mortality curves which support the concept proposed here that there is a factor(s) present in the brains of female mice which enhances the mortality rate of male and female animals and that this factor is neutralized partially by some factor present in the brains of male animals. These postulated factors were shown to be present in animals whether they were infected with encephalomyocarditis virus or were normal and apparently healthy. Detailed data in support of this concept were presented.

REFERENCES