The Mouse Inoculation Test in Rabies Diagnosis: Early Diagnosis in Mice During the Incubation Period

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ABSTRACT

Brain tissue from 64 rabies suspect specimens were inoculated intracerebrally into twenty 9-12 gm adult Swiss white mice. Two mice from each specimen were killed on specific days postinoculation and examined for the presence of rabies virus by the fluorescent antibody staining technique. In this way a positive diagnosis was made in the majority of cases between postinoculation days 4 and 12 when the incubation period of these same specimens ranged between eight and 20 days.

RESUME

Après avoir préparé une émulsion du cerveau de chacun des 64 animaux soupçonnés de rage inclus dans cette expérience, les auteurs inoculèrent autant de groupes de 20 souris blanches, dont le poids variait entre neuf et 12 grammes. Ils sacrifièrent deux souris de chacun des groupes à des dates spécifiques après leur inoculation et procédèrent à la recherche du virus rabique dans leur cerveau, à l'aide de l'immunofluorescence. De cette façon, ils réussirent à poser un diagnostic de rage, dans la plupart des cas, entre le quatrième et le 12e jour après l'inoculation, alors que la période d'incubation de ces cas dura de huit à 20 jours.

With the introduction of the fluorescent antibody staining technique (FAT) for the diagnosis of rabies the proportion of specimens initially FAT negative and subsequently proven to be rabies positive by mouse inoculation techniques (MIT) is small. At this laboratory there have been 58 such cases (0.6%) in some 9,670 specimens inoculated into mice during the past three years. However, the delay in diagnosis in these cases poses a serious public health problem.

The World Health Organization Expert Committee on Rabies (3) recommends that sufficient animals be inoculated to allow for the daily euthanasia of one or two of these mice in an attempt to demonstrate rabies antigen in the brains before clinical signs are seen or death occurs. There have been few reports of studies to elucidate this question and fewer recommendations as to the exact procedures to be used (2, 4, 5, 6). The present study was undertaken in an attempt to establish some feasible system for a laboratory which handles approximately 3,000 cases involving human contact and an equal or greater number of cases not involving contact per year.

Infected brain tissue was obtained from 64 field cases (Table I) submitted to this laboratory and was chosen because of a poor staining reaction with the FA technique. Ten of these specimens were originally diagnosed as rabies negative by the FAT but were MIT positive. A 10% brain suspension in physiological saline was inoculated intracerebrally into twenty 9-12 gm adult Swiss white mice (0.03 ml/mouse).

Two examination schedules were followed. In the first, beginning with postinoculation day 2 and every second day thereafter, two mice were killed, impression smears of a slice of brain to include diencephalon, midbrain, pons, medulla, cerebellum and brain stem were prepared and stained by the FA technique (1). The second schedule differed only in that mice were killed on postinoculation day 4 and every day thereafter. Once a specimen had been demonstrated to be rabies positive in this way, all remaining mice were left for

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the disease to run its course and the first death was noted. No discrepancies could be demonstrated between the two schedules of killing mice except that those found positive on any given alternate day in the first trial could possibly have been found positive one day sooner had they been so tested.

Figure 1 illustrates the comparison between the postinoculation day on which the first mouse was diagnosed rabies positive by sequential euthanasia and the first day on which a diagnosis could be made after natural death. In 44 of the cases, both mice were rabies positive, while in the remaining 20 only one of the mice killed was diagnosed as positive. The majority of cases could be diagnosed as positive between the fourth and eighth day postinoculation by sequential euthanasia. However, when allowed to develop the disease and die, a positive diagnosis could not be obtained until a period between the eighth and sixteenth postinoculation days. In 31 cases, a diagnosis could be made in one half the time or less of that required for the conventional MIT. In the remaining cases, the time required for a diagnosis was between one half and three quarters the time of the MI test. In general, the longer the incubation period of a specimen in mice the longer the period between inoculation and diagnosis after euthanasia.

It has been noted that specimens which are FAT negative and MIT positive usually have an extended incubation period in mice. This fact is illustrated in Fig. 2 from results obtained in other studies (unpublished data) at this laboratory. This was also demonstrated in the present study and with one exception all specimens which were initially FAT negative had an incubation period of 13 days or more (up to 33 days). When mice from these specimens were sequentially examined, they were all found rabies positive between postinoculation days 6 and 12. In six of these ten specimens a diagnosis could be made in half the time or
Fig. 2. Comparison between incubation periods in mice of specimens which were FAT positive/MIT positive and of specimens FAT negative/MIT positive.

TABLE I. Rabies Diagnosis by Sequential Euthanasia of Experimental Mice: Species and Number of Specimens

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Specimens Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mice Killed every 2nd day</td>
</tr>
<tr>
<td>Cat</td>
<td>2</td>
</tr>
<tr>
<td>Dog</td>
<td>5</td>
</tr>
<tr>
<td>Bovine</td>
<td>8</td>
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<tr>
<td>Horse</td>
<td>8</td>
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<td>Sheep</td>
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<tr>
<td>Bat</td>
<td>1</td>
</tr>
<tr>
<td>Fox</td>
<td>3</td>
</tr>
<tr>
<td>Skunk</td>
<td>1</td>
</tr>
</tbody>
</table>

There have been several studies employing relatively small numbers of specimens and various age groups of experimental mice which have demonstrated that early diagnosis is possible. Goldwasser and
Kimron (2) used tissues from seven dogs, one cat and one jackal. Lodmell et al (4) used a bat virus. Moron et al (6) inoculated tissues from 11 dogs and one cat. Markson et al (5) inoculated tissue from three dogs. Specific fluorescence was demonstrated between three and 11 days postinoculation. The present study supports these results and emphasizes the fact that a positive diagnosis by MIT on specimens which are FAT negative can be obtained well in advance of clinical signs and/or death of experimental mice.

From the results of this study it is recommended that, where the mouse inoculation test is used as an adjunct to the fluorescent antibody staining technique in cases where the offending animal is a known hazard to humans and severe exposure has occurred, every attempt should be made to inoculate sufficient mice to enable the sequential killing and examination of these mice. The above results indicate that the time required to diagnose these cases could be substantially reduced by sequentially killing and examining mice beginning at the fourth day following inoculation.

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REFERENCES