Simple Method of Evaluating Scrapie in Mice

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Mice in advanced scrapie characteristically clasp their hindlegs together. As the disease progresses into the clasp stage, mice exhibit a sequence of identifiable hindleg reactions which have categorized and assigned relative scores permitting objective, quantitative evaluation of disease.

The establishment of scrapie in mice by Chandler (1) has provided a practical, maneuverable, and relatively inexpensive laboratory animal model for studying slow virus infections. Scrapie is a chronic, degenerative neurological disease lasting 2 to 5 years in sheep, its natural host, and 4 to 12 months in laboratory mice. Its relevance to human disease was recognized by Hadlow (2), who noted a similarity between kuru in man and scrapie in sheep. Scrapie, kuru, mink encephalopathy, and Creutzfeldt-Jakob disease have been classed together as the subacute spongiform viral encephalopathies (3). Moreover, this group of disorders bears striking similarities to such diseases of man as multiple sclerosis, Parkinsonism, and amyotrophic lateral sclerosis (4). As noted by Chandler (1) and confirmed by others (3, 11), mice in advanced scrapie, when suspended by the tail, characteristically clasp their hindlegs together, whereas normal mice splay theirs outward. We observed that, in the development of scrapie, mice exhibit a sequence of responses before the clasp reaction is manifest. This sequence of responses has been categorized, and the stages have been assigned relative scores to provide a quantitative index of the disease in mice.

MATERIALS AND METHODS

The Chandler strain of mouse-adapted scrapie was obtained under a U.S. Department of Agriculture permit from W. J. Hadlow. Its history has been described (3). In these experiments, 0.03 ml of 1% brain suspension in saline of the sixth mouse passage was inoculated intracerebrally into Swiss Webster mice, either purchased from Spartan Laboratories or their laboratory-bred descendants. To evaluate disease, a mouse is picked up by the tip of its tail and suspended in midair for approximately 5 sec. This period of suspension is too brief for the animal to become distressed and climb up to its own tail. It is, however, long enough for the clasp reaction to be displayed in a sufficiently affected mouse. In the normal response, scored 0, the hindlegs are splayed more or less horizontally, and the mouse may twist about in mild protest. In the earliest disease category, scored 1, the animal accepts being suspended much more placidly, and the hindlegs are held at angles between 45 and 90° below horizontal, usually symmetrically. The bases for distinguishing the first category from the normal are the angles of the hindlegs (depressed 45° or more) and the departure of the animal. In the next more severe category, scored 2, the hindlegs are held parallel with surprising precision and constancy, usually pointed vertically downward. Also included in this category are those mice which proceed to retract one leg against the abdomen in a unilateral clasp action. In the most advanced stage of disease, scored 3, as previously noted (1, 3, 11), the suspended mouse rather promptly clasps the hindlegs together, frequently against the abdomen. The toes may be interlocked, the feet may be held together with the toes clenched, or the toes of one foot may be wrapped around the other clenched foot. Untreated disease is uniformly fatal, and, for cumulative scoring, death is rated 4. These categories and their characteristics are summarized in Table 1.

RESULTS

The disease produced in mice was by gross and microscopic observations compatible with descriptions of mouse scrapie. The clinical features of scrapie, noted by others (1, 3, 11), were also seen in our mice, including changes in gait, posture, appearance, and behavior. None seemed as definite or as amenable to simple objective evaluation as the hindleg response, which may be considered pathognomonic. No particular foreleg responses have been reported (1, 3, 11) or noted by us. Control mice, including uninoculated mice in the same room or mice inoculated with equal volumes of 1% normal brain suspension, buffered saline, or plain broth and observed for comparable intervals, remained normal. Although some nonspecific deaths would be anticipated, none occurred in these particular groups, and all mice inoculated with 1% scrapie brain material died with scrapie, as judged clinically. Histopatho-
logical examination of representative animals confirmed the disease.

Currently, data for complete courses of disease are available only for mice inoculated intracerebrally. Results of scoring a typical group of affected mice throughout the disease are shown in Fig. 1. In addition to the cumulative average scores, ratings of individual animals are presented in scattergram form. Any scheme of grading a uniformly fatal condition giving greatest weight to death would exhibit an increasing cumulative score as deaths increase, without regard to the rating of intermediate stages. These data show that the intermediate stages of this system, particularly categories 1 and 2, are rated in realistic sequence. Since the mice were rated periodically, it is possible to calculate the interval to each category of disease severity. Table 2 gives the median intervals required for several groups of 15 mice each to reach the various stages and shows the relative uniformity of response. Application of this rating system to other experimental neuropathies and correlation with neuronal changes are being studied.

**DISCUSSION**

Systems of quantitating neurological disease in man have been proposed, particularly with regard to the objective evaluation of therapy (10). Current interest and activity in the treatment of chronic neuropathies (2, 5, 9, 14) point up the need for usable preclinical models. Indeed, in the United States, there is a legal obligation to seek evidence of efficacy in other systems prior to testing new therapies in man (Code of Federal Regulations, Title 21, Part 130.3). The relation of scrapie to other neurological diseases has been cited (6–8). Scrapie can be measured in vivo by observing when and how many inoculated animals die. In addition, quantitative cytological analyses of lesions can be made (4, 12, 13), but such examinations involve sacrificing the animals. Analysis of the behavior of scrapie mice by the open-field method (13) requires special equipment and more time and, by its nature, is less desirable for an infectious disease. In contrast, the hindleg response and the reactions preceding it offer a simple but objective and quantitative way to evaluate the occurrence and progress of scrapie in mice while preserving them for further observation. The hindleg response, therefore, seems especially suitable for evaluating the course of the disease and the response to experimental treatment.

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