Characteristics of a Short Incubation Model of Scrapie in the Golden Hamster

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(Accepted 14 September 1976)

SUMMARY

Repeated passage of the ‘Chandler’ strain of scrapie in female golden hamsters using the intracerebral route of inoculation reduces the minimum incubation period to 60 days, about half of the minimum incubation period so far found in any of the mouse models of scrapie. The infectivity titres in brain in the clinical stage of the disease are considerably higher ($> 8 \times 10^{-10} \text{LD}_{50}$ i.c. units/0.05 g) than those found in mouse scrapie. The biological characteristics of this model of hamster scrapie are reported, including the effects on incubation period of route of inoculation, dose of agent, sex of hamster, ambient temperature (hibernation) and splenectomy. Some general and specific applications of this experimental model of scrapie are discussed.

INTRODUCTION

The first transmission of scrapie to golden hamsters was reported by Zlotnik & Rennie (1965) who used the ME7 strain of agent. After the third hamster passage, the incubation period following intracerebral (i.c.) injection with high doses of agent was 4 months; about the same as the shortest incubation period achieved with any combination of agent-strain and mouse-strain. Chandler & Turfrey (1972) described the transmission of ‘Chandler’ scrapie to Chinese hamsters with a minimum incubation period of 4 months after serial passage. They also showed that the incubation period of ‘Chandler’ scrapie in bank voles could be as short as 2 months but this host species has not been widely used in scrapie research. In the same series of experiments Chandler & Turfrey transmitted scrapie to the golden hamster but the incubation period at first pass was 11 months. At second pass, the incubation period dropped to 4 to 5 months (see Fig. 1) and animals at second passage were used in a comparative study of ‘Chandler’ scrapie with transmissible mink encephalopathy (TME) in the golden hamster (Kimberlin & Marsh, 1975; Marsh & Kimberlin, 1975). These studies also revealed exceptionally high titres of scrapie infectivity in the brains of clinically-affected hamsters.

We have now completed the ninth serial passage of ‘Chandler’ scrapie in golden hamsters and found that, after the fourth passage the incubation period stabilizes at 60 days following i.c. injection of high doses of agent. This is about half of the minimum incubation time found in mice. The present paper describes the characteristics of this potentially useful model of scrapie.
METHODS

Source of scrapie. The scrapie agent used was that originally derived from the Compton ‘drowsy goat’ source and transmitted to mice by Chandler (1963). After three passages in mice it was transmitted to rats (Chandler & Fisher, 1963) and passed several times before transmission to hamsters (Chandler & Turfrey, 1972). The hamster passaged agent is designated the ‘Chandler’ strain but it is not necessarily the same as the ‘Chandler’ strain passaged in mice. To date, three agent strains have been isolated from the ‘drowsy goat’ source (Dickinson, 1976, Fig. 10.2) and preliminary data (Kimberlin & Fraser, unpublished data) suggest that the agent strain isolated from the second hamster passage, and passaged in mice, is different from the main line of ‘Chandler’ agent passaged repeatedly in mice (also known as 139A). The present studies were carried out using two half-brains from first pass in golden hamsters (Chandler & Turfrey, 1972), one from an animal that showed clinical signs and histological lesions of scrapie 11 months after i.c. inoculation, and one from a clinically normal animal which had histological lesions of scrapie when killed after 12 months.

Animals. Most experiments were carried out using out-bred golden hamsters from a local supplier. Except where otherwise stated, females only were used and animals were inoculated at 6 weeks of age. Splenectomized and sham-operated animals were purchased from Charles Rivers U.K. Ltd., Margate, Kent. All animals were kept singly in plastic ‘mouse’ boxes with stainless steel lids and given water and a pellet diet *ad libitum*.

Inocula. Apart from titrations of infectivity within spleen, all inocula were prepared from brains taken from clinically-affected hamsters. Animals were killed with chloroform. Tissues were stored frozen at –20 °C in new glass bottles and were homogenized in sterile saline using Teflon-glass, Potter-Elvejhem homogenizers. All instruments and homogenizers were given one precleaning and two postcleaning cycles of autoclaving at 20 psi (126°C) for 30 min. Homogenates were prepared at a wet weight concentration of 5 or 10%. Dilutions of uncentrifuged suspensions were prepared in saline using disposable plastic-tipped pipettes and new glass bottles. In all cases the vol. of inoculum was 0.05 ml. Unanaesthetized animals were injected intraperitoneally (i.p.) into the lower left quadrant. Light, ether anaesthesia was employed for i.c. inoculations directly into the mid-brain, just to the right of the mid-line. All inoculations were done with plastic disposable syringes fitted with a 26G needle. Titrations of infectivity were carried out by injecting groups of 4 hamsters with serial 10-fold dilutions of inocula by the i.c. route and observing the development of disease. End points were calculated according to the method of Kärber (Parker, 1959) and refer to the amount of infectivity in 0.05 g of tissue. Infectivity titres/g of tissue can be calculated by adding \(1.3 - \log_{10} \text{LD}_{50}\) units. Passage experiments were performed with 1, 5 or 10% homogenates inoculated into groups of 4 to 6 hamsters except where otherwise indicated.

Measurement of incubation period. Animals were examined at least twice a week. Early clinical signs of scrapie include destruction of nest (made from shredded paper) and hypersensitivity to noise and sudden movement. Animals were scored as positive when unmistakable signs of uncoordinated movement had developed, about 5 to 10 days after the earliest behavioural changes. Scrapie-affected hamsters were either dead or moribund two weeks after being scored positive. In critical experiments, only coded information was displayed on hamster boxes to avoid observer bias. All survivors were kept until 300 days after inoculation.
Scrapie in hamsters

Fig. 1. Passage history of ‘Chandler’ scrapie in golden hamsters. Two pools of 2nd pass material were set up, each pool containing two half-brains from two animals killed 147 days after infection. All passes were made with brain homogenates (usually 1, 5 or 10 %) using the i.c. route of inoculation. Mean incubation periods are given in days ± s.e. of 4 to 6 hamsters. Scrapie agent from both pass lines was cloned by passaging 10⁻⁶, 10⁻⁷ or 10⁻⁸ dilutions of brain homogenates from clinically-affected hamsters i.e. at the titration end point (see Table I). (a) and (b) refer to replicate passes carried out after an interval of 5 months (Line 1) or 16 months (Line 2).

RESULTS

Passage of ‘Chandler’ scrapie in golden hamsters

Brain material from the first pass of ‘Chandler’ scrapie in golden hamsters was passaged a second time, both in Madison (Kimberlin & Marsh, 1975; Marsh & Kimberlin, 1975) and also at Compton (Fig. 1), to give a much reduced incubation time (110 to 140 days) after i.c. infection with low dilutions of scrapie brain. Two separate passage lines were then established at Compton and in both lines the incubation period decreased even further at 3rd, 4th and 5th passes (Fig. 1). Thereafter, the incubation period stabilized to give an average of 60 days at 5th to 9th passages with 5 to 10 % brain inocula and an average of 71 days with 1 % brain inocula.

Infectivity titres in scrapie brain

The titres of infectivity in the brains of clinically-affected hamsters at each passage level are shown in Table 1. Titres ranged from 7·2 to 8·9 -log₁₀ LD₅₀ i.c. units/0·05 g but it is interesting to note a tendency in both passage lines for titres at earlier passes to be higher than at later passes. The mean of the 12 values shown in Table 1 is 8·4 -log₁₀ i.c. units. This figure compares with values in the range of 7·0 to 7·5 i.c. units/0·03 g routinely found in the brains of mice clinically affected with ‘Chandler’ scrapie when titrated in an identical manner.
Table I. *Titration of infectivity in hamster brain at different passage levels*

<table>
<thead>
<tr>
<th>Passage level</th>
<th>Line 1</th>
<th>Line 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PID†</td>
<td>Titre‡</td>
</tr>
<tr>
<td>Third</td>
<td>91</td>
<td>8'9</td>
</tr>
<tr>
<td>Fourth Pool</td>
<td>8'3</td>
<td>98</td>
</tr>
<tr>
<td>Fourth 103-147</td>
<td>7'8</td>
<td>149</td>
</tr>
<tr>
<td>Fifth Pool</td>
<td>7'8</td>
<td>142</td>
</tr>
<tr>
<td>Fifth 54-61</td>
<td>7'2</td>
<td></td>
</tr>
<tr>
<td>Sixth</td>
<td>53</td>
<td>7'9</td>
</tr>
<tr>
<td>Seventh</td>
<td>73</td>
<td>230</td>
</tr>
<tr>
<td>Eighth</td>
<td>127</td>
<td>8'0</td>
</tr>
</tbody>
</table>

* Single or pooled half-brains were titrated, taken from intracerebrally infected hamsters with clinical scrapie.
† PID, days after infection when brains were taken.
‡ Titre, -log₁₀ LD₉₀ i.c. units/₀·₀₅ g brain.
§ ND, not done.

Some factors affecting incubation period

Hamster strain

Studies of golden hamsters have been carried out at Compton for 5 years using only outbred hamsters from a single supplier. There has been no evidence from the range of incubation periods in groups of hamsters given the same inoculum that animals from this source are segregating for a gene equivalent to the sinc gene which has a major effect in controlling the incubation period of scrapie in mice (Outram, 1976).

Age of hamsters

The present studies have not revealed any marked age-effect on incubation time in hamsters infected between 6 weeks and 10 months of age. We have not investigated whether infection of younger hamsters produces a proportion of animals with abnormally short or long incubation periods (compared to adults) such as occurs in neonatal mice (Outram, Dickinson & Fraser, 1973; R. H. Kimberlin, unpublished results). In one experiment, the infectivity titre in the brain of a clinically-affected hamster (Line 2, 7th pass) was estimated in hamsters 24 days old and 100 to 140 days old at the time of inoculation. The titres obtained (8'5 and 8'2 -log₁₀ LD₉₀ i.c. units/₀·₀₅ g respectively) were not significantly different in view of the small group size (4 hamsters per dilution) used in titrations.

Sex of hamster

All the studies described in this paper have used female hamsters. In one experiment involving the inoculation of a 5% homogenate of scrapie brain (Line 2, 3rd pass) into groups of 8 hamsters it was found that the incubation period was significantly longer in male hamsters than in females; by 8% using the i.c. route of inoculation and 16% using the i.p. route. This finding agrees with the similar, though not invariable, sex-effect found with many strains of scrapie in mice (Outram, 1976).
Scrapie in hamsters

Table 2. Effect of ambient temperature on incubation time in i.c. or i.p. infected hamsters*

<table>
<thead>
<tr>
<th>Dilution of scrapie brain</th>
<th>Route of inoculation</th>
<th>‘Hot group’ Incubation period in days (mean ± s.e. of (n) hamsters)</th>
<th>‘Cold group’ Incubation period in days (mean ± s.e. of (n) hamsters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁶</td>
<td>i.c.</td>
<td>106±3 (17)</td>
<td>106±2 (14)</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>i.p.</td>
<td>102±3 (15)</td>
<td>104±4 (11)</td>
</tr>
</tbody>
</table>

Temperature °C (mean ± S.D.)

<table>
<thead>
<tr>
<th>Mean minimum</th>
<th>Mean maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.6±2.9</td>
<td>12.7±2.3</td>
</tr>
<tr>
<td>23.8±3.0</td>
<td>14.8±2.4</td>
</tr>
</tbody>
</table>

* The scrapie inoculum was prepared from clinically-affected hamsters from Line 2, 7th pass (see Fig. 1). Immediately after infection, animals were kept in ‘hot’ or ‘cold’ rooms for 90 days (November to February) and then both groups were maintained at the temperature of the ‘hot’ room. The temperatures shown are the means of daily readings for the 90-day period.

Ambient temperatures

Early studies revealed practical problems in measuring incubation times in golden hamsters because of their tendency to short periods of hibernation during the winter months unless temperature and lighting conditions are rigidly controlled. This observation led us to consider the possible effects of hibernation on incubation time per se under conditions where none of the hamsters hibernated during the time when clinical signs were developing. About half of the i.c. and i.p. infected groups kept in the cold (13 to 15 °C) underwent short periods (2 to 4 days) of hibernation but this had no significant effect on incubation period (Table 2). However, there was one hamster from the i.p. inoculated, ‘cold’ group which went into hibernation for most of the last 42 days of exposure. The incubation period in this hamster was excessively long, being 153 days and more than 3 standard deviations outside the mean (104 days) for the rest of the group.

Infection by the intraperitoneal route

The efficiency of the i.p. route of infection in producing disease can be gauged from the ratio of incubation times after injection of the same dose of a standard inoculum by the i.p. and the i.c. route. A total of five determinations of the i.p./i.c. ratio were made with 0.05 ml samples of 1, 5 or 10% brain homogenates from clinically affected hamsters taken at 3rd and 4th passage of scrapie. The range was 1.8 to 2.3 compared to values in the range of 1.2 to 1.4 for ‘Chandler’ scrapie in three different mouse strains (R. H. Kimberlin, unpublished data). These results suggest that the i.p. route is relatively inefficient in hamsters, at least for the ‘Chandler’ strain of scrapie. This conclusion was confirmed by the results of two titrations of infectivity of hamster brain (Lines 1 & 2, 4th pass). The infectivity titres (−log₁₀ LD₅₀ units/0.05 g) were 8.7 i.c. units and 4.0 i.p. units in one experiment and 8.3 i.c. units and 3.8 i.p. units in the other; a difference between routes of inoculation of 4.7 units and 4.5 units, respectively.

Other experiments have been carried out with 5th and 6th hamster passage of TME (Sawyer County isolate; Kimberlin & Marsh, 1975) in which i.p. inoculations of 1% or 5% homogenates of clinically-affected brain failed to produce 100% cases of TME in golden hamsters. These findings indicate that the inefficiency of the i.p. route may be a general feature of hamsters infected with scrapie and related agents.
Table 3. Effect on incubation time of splenectomy before intracerebral or intraperitoneal infection with scrapie*

<table>
<thead>
<tr>
<th>Dilution of scrapie brain</th>
<th>Dose of agent (LD₅₀ units)</th>
<th>Route of infection</th>
<th>Incubation period in days (mean ± s.e. of (n) hamsters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁴</td>
<td>180 i.c. units</td>
<td>i.c.</td>
<td>Sham operated: 107 ± 2 (8) Splenectomized: 106 ± 2 (8)</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>560 i.p. units</td>
<td>i.p.</td>
<td>Sham operated: 112 ± 5 (9) Splenectomized: 118 ± 3 (9)</td>
</tr>
<tr>
<td>10⁻²</td>
<td>56 i.p. units</td>
<td>i.p.</td>
<td>Sham operated: 141 ± 6 (9) Splenectomized: 130 ± 5 (9)</td>
</tr>
</tbody>
</table>

* The scrapie inoculum was prepared from clinically-affected hamsters from Line 1, 4th pass (see Fig. 1).

Infectivity titres in spleen

Marsh & Kimberlin (1975) reported titres of infectivity in hamster spleens in the preclinical and clinical stages of disease, following i.c. infection with brain homogenates from the 2nd pass of 'Chandler' scrapie in hamsters. Titres ranged from 2·3 to 4·3 \(-\log_{10} LD₅₀\) i.c. units/0·05 g of spleen. These values are relatively low compared to the maximum titres of 5·0 to 6·0 \(-\log_{10} LD₅₀\) i.c. units/0·05 g of mouse spleen after i.c. and also i.p. infection with 'Chandler' scrapie (Clarke & Haig, 1971; Hunter, Kimberlin & Millson, 1972). In the present study, infectivity titres were measured in spleens from clinically-affected hamsters following i.p. infection with 3rd and 6th passage of 'Chandler' scrapie to give values of 6·0 and 5·5 \(-\log_{10} LD₅₀\) i.c. units/0·05 g of spleen, respectively. These values are higher than those found after i.c. infection and indicate that multiplication of agent in the spleen may be as important in the pathogenesis of peripherally injected scrapie in hamsters as it appears to be in mouse scrapie (at least with the ME7 and 'Chandler' strains of agent: Fraser & Dickinson, 1970; Clarke & Haig, 1971; Dickinson & Fraser, 1972).

Effects of splenectomy

The importance of the spleen in the pathogenesis of i.p. injected hamster scrapie was investigated further by studying the effects of splenectomy. As expected, surgical removal of the spleen before i.c. infection has no effect on incubation time (Table 3). However, splenectomy before i.p. infection had no effect either at the two dose levels of agent used. It is important to note that because of the inefficiency of the i.p. route of infection with 'Chandler' scrapie in hamsters, the maximum infecting dose was only 560 i.p. units (Table 3).

Dose response curves

Fig. 2 illustrates the regular relationship between incubation time and dose of agent (dilution of scrapie brain) following i.c. infection of hamsters. In comparison to the i.c. route, the incubation periods following i.p. injection of 10⁻¹ and 10⁻² dilutions of brain are much longer. However, it is important to note that when allowance is made for the inefficiency of the i.p. route by plotting the number of i.p. infectious units against incubation period, then the i.c. and i.p. curves are much closer together (Fig. 2). These results show that the incubation period following injection of 10 to 100 i.p. infectious units is very similar to that found after injecting 10 to 100 i.c. infectious units (see also Table 4, lines 2 and 4).
Scrapie in hamsters

Fig. 2. Relationship between dose of agent and length of incubation in i.c. and i.p. infected hamsters. The scrapie brain inoculum was prepared from clinically-affected hamsters from Line 1, 4th pass and contained $8.3 - \log_{10} \text{LD}_{50}$ i.c. units/0.05 g of brain and 3.8 i.p. units. The solid line (●) shows the incubation time in hamsters injected i.c. with different dilutions of scrapie brain, shown on the left-hand abscissa. The origin of the right-hand abscissa is adjusted so that the same line relates incubation time to the number of infectious units (by the i.c. route). The incubation period of hamsters injected i.p. with $10^{-1}$ and $10^{-2}$ dilutions of scrapie brain is shown by ▼. The same data are also plotted against the number of infectious units (by the i.p. route) and are shown by ▽. Bars represent s.e.

Table 4. Comparison of the amount of agent in brain in clinically-affected hamsters following infection with different doses of agent by different routes of inoculation

<table>
<thead>
<tr>
<th>Dilution of scrapie brain*</th>
<th>Dose of agent ($-\log_{10} \text{LD}_{50}$ units)</th>
<th>Route of infection</th>
<th>No. of brains assayed</th>
<th>No. of recipient brains per donor brain</th>
<th>Incubation period (days) of (n) recipients (mean ± s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-4}$</td>
<td>6.3 i.c. units</td>
<td>i.c.</td>
<td>3</td>
<td>4</td>
<td>93 ± 1 (12)</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>≥2 i.p. units</td>
<td>i.p.</td>
<td>3</td>
<td>4</td>
<td>96 ± 2 (12)</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>6.3 i.c. units</td>
<td>i.c.</td>
<td>2</td>
<td>3</td>
<td>93 ± 1 (6)</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>1.3 i.c. units</td>
<td>i.c.</td>
<td>2</td>
<td>4</td>
<td>93 ± 1 (8)</td>
</tr>
</tbody>
</table>

* In experiment 1, donor animals were infected with scrapie brain from Line 2, 5th pass and in experiment 2 with scrapie brain from Line 2, 6th pass. Homogenates of each donor brain were diluted to $10^{-5}$ and injected i.c. into recipient hamsters.

Effect of different conditions of infection on the amount of infectivity in clinically-affected hamster brain

It has been suggested, from the general features of scrapie pathogenesis in the mouse, that the sharp onset of clinical disease is related to the attainment of a certain critical concentration of agent in the CNS (Kimberlin, 1976a, b). Two simple experiments were carried out to
test this suggestion in hamster scrapie. Donor hamsters were infected with different doses of agent by the i.c. or i.p. routes. When clinical signs developed, $10^{-5}$ homogenates of donor brains were injected i.c. into groups of recipient hamsters and the incubation times were measured. All four recipient groups showed the same incubation period (to within 3 days; Table 4). From the dose-response relationship illustrated in Fig. 2 it can be calculated that the average concentration of agent in the various donor brain groups did not vary by more than 0·1 to 0·2 $-\log_{10} LD_{50}$ i.c. units.

**DISCUSSION**

Our original reason for carrying out scrapie experiments in golden hamsters was to make a detailed comparison between scrapie and TME in a common host species, the golden hamster being the only one readily available (Kimberlin & Marsh, 1975; Marsh & Kimberlin, 1975). In the course of this work it was found that many of the biochemical changes that occur in the brains of scrapie-affected mice were not found in hamster scrapie. Later, we made comparative studies of scrapie in mice, rats, golden hamsters and Chinese hamsters and found this to be an invaluable way of assessing the primary significance of biochemical events in the development of scrapie (Kimberlin, Collis & Walker, 1976).

The present paper describes the characteristics of ‘Chandler’ scrapie in hamster. We suggest that this scrapie model may be of general use in checking the validity of key findings from studies of mouse scrapie. We also suggest that ‘Chandler’ scrapie in hamsters offers some unique advantages to scrapie research, studied alone or in addition to some of the models of mouse scrapie.

The onset of clinical signs of hamster scrapie is very sharp which makes it relatively easy to measure the length of incubation period. The accuracy of this measurement is not as precise as is commonly found in random-bred mice but biological experiments can be performed with groups of 6 to 10 out-bred hamsters to give a s.e. of less than 5% of the total incubation time (see Tables 3 and 4).

Two particular advantages of this hamster scrapie model are the high infectivity titres in brain in the clinical stage of the disease, and the very short incubation period after i.c. infection. Although surviving hamsters were observed for 300 days to allow time for occasional late cases to develop after very low doses of infective agent, in practice the essential result of a titration can often be obtained in 150 to 200 days.

The availability of both hamster and mouse models of scrapie is invaluable in studies of the so-called species barrier (see Kimberlin, Walker & Millson, 1975; Dickinson, 1976). Assays of infectivity can be carried out simultaneously in both species to study the ‘adaptation’ of scrapie from one species to another. In the present study, the reduction of the i.c. incubation time in golden hamsters, from $>300$ days at 1st pass to 60 days at 5th and subsequent passes, is a striking example of this kind of phenomenon which we are currently investigating. The results of some preliminary experiments, involving the transmission of hamster passaged scrapie (at different passage levels) to mice suggest that the decreased incubation time may be partly due to selection, from a mixture of 2 or more strains, of an agent which is highly pathogenic for golden hamsters. These studies have also revealed an additional way of identifying different strains of scrapie agent (Dickinson, 1976) by simultaneously comparing their biological properties in hamsters and mice. Details of this work will be published later.

In contrast to the short incubation times and high infectivity titres obtained in hamsters infected by the i.c. route, the i.p. route of inoculation is remarkably inefficient; much more so than is commonly found with a number of the mouse models of scrapie where different
routes of inoculation have been compared (A. G. Dickinson & R. H. Kimberlin; unpublished data). It is clear that most of the infectivity injected i.p. into hamsters is either actively degraded or removed in some other ways, and we suggest that this may be a useful experimental system in which to study the inactivation of scrapie agent in vivo.

The ability of hamsters to hibernate offers an interesting way of investigating the control processes exerted by the host on the multiplication of scrapie agent in vivo (Dickinson & Fraser, 1976; Outram, 1976). The extended i.p. incubation time in a solitary hibernating hamster may be compared with the shortened incubation time of scrapie (i.c. injected, ME7 agent) in BALB/c mice given thyroxine in their drinking water (Fraser, in Outram, 1976). These preliminary results suggest that the metabolic activity of certain (as yet unknown) types of cell may be important to the multiplication of scrapie agent. It is interesting to note that the incubation period of rabies in bats can be considerably lengthened by hibernation (Sadler & Enright, 1959).

The role of the spleen in i.p. injected hamster scrapie is uncertain from the results described. Reasonably high titres of infectivity are found in the spleens of clinically-affected hamsters suggesting that agent can multiply in this organ after i.p. infection; but splenectomy has no effect on incubation time (Table 3), a finding which appears to conflict with the delayed i.p. incubation time of scrapie in splenectomized mice (Fraser & Dickinson, 1970; Clarke & Haig, 1971; Dickinson & Fraser, 1972). However, these results in mice were obtained using relatively large doses of agent. It has been found that whereas splenectomy prolongs the incubation of high doses of ME7 agent, injected i.p. into C57BL mice, it has no effect on the incubation time of low doses (Fraser & Dickinson, 1970, and personal communication). Fraser & Dickinson conclude that the pathogenesis of low doses of scrapie is unaffected by splenectomy because sufficient non-splenic extraneural sites are present for both the uptake and multiplication of these low doses of agent. This interpretation is consistent with the finding that spleen is not the only site of extraneural multiplication of scrapie agent (Eklund, Kennedy & Hadlow, 1967; Outram, 1976). The failure, in the present experiments, of splenectomy to alter the incubation time in scrapie-infected hamsters could be explained by the inefficiency of the i.p. route which reduces the amount of infecting agent to below the level at which the presence of the spleen is a limiting factor in pathogenesis.

We gratefully acknowledge the expert care of the animals provided by Janice A. Robinson and Janet C. Simmons.

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(Received 26 July 1976)