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# A comparison of some biological characteristics of the mouse-passaged scrapie agents, 22A and ME7

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## 1. INTRODUCTION

Scrapie is a fatal neuropathic infection, with an incubation period ranging from 5 months to several years, which occurs naturally in sheep where it is transmitted vertically from mother to offspring and, also, laterally by some infectious or contagious process, the extent of which is uncertain. It has been transmitted experimentally to sheep, goats, mice, rats and hamsters. In some cases there are clear-cut clinical signs of neurological damage, with ataxia, pruritis and emaciation, but in other cases the disease follows an insidious course with clinical signs which are not diagnostic: however, there are characteristic lesions in the brain involving neuronal vacuolation, status spongiosus and astrocytic hypertrophy. Diagnosis is made on the combined clinical and histological findings. The transmissable agent, which is considered to be less than 25 m$\mu$ in size, replicates in the host but it has not been isolated, concentrated or grown in tissue culture; and doubt has been cast on whether it can be a virus, conventionally based on nucleic acid, because of its extreme resistance to physical or chemical inactivation. It has been shown to withstand boiling, repeated freezing and thawing, ultraviolet and ionizing irradiation, formaldehyde, DNase, RNase, proteolytic enzymes, $\beta$-propiolactone and pH's ranging from 2·5 to 10·5. A recent review of work on scrapie has been given by Stamp (1967).

The two strains of scrapie agent to be described differ in terms of the disease process, particularly in incubation period but also in the distribution of lesions in the brain and the detailed course of the disease, but no attempt has been made to detect any physical or chemical differences. These biological characteristics of each agent persist on serial passage in different genotypes of mice, indicating accurate copying during replication and, if the agents were not known to have such unusual physical and chemical properties, it would be reasonable to assume that DNA or RNA was involved. If it is eventually shown that no nucleic acid is involved then some other basis for the transmission of genetic information must exist.

Evidence for the difference between the two agents used principally concerns incubation period. The incubation period of scrapie may, however, vary for a number of reasons, so that a simple difference in incubation period does not necessarily indicate a qualitative difference between agents from different sources. Among the many factors which can affect incubation period are dose and titre,

concordance between the species of agent-donor and recipient and the previous passage history of the agent, certain physical or chemical treatments of the inoculum, the route of administration and, for some routes, the age of the recipient, and the host's genotype. The ME7 and 22A agents differ in passage history and could also differ to a small extent in the titres used. After allowing for these factors we aim to show that important qualitative differences remain.

Although scrapie agents with different properties have previously been found (Pattison, 1966; Pattison & Jones, 1968) no reports have been published of the type given here, in which mouse strain differences in response to one agent are reversed for the other agent, nor has any scrapie agent previously been reported for which the incubation period following a high challenge dose is of the same order as the normal lifespan of the mouse.

## 2. MATERIALS AND METHODS

### (i) *Mouse stocks*

The stocks $SS_x$, $LS_x$ and F, which are different highly inbred closed populations, were bred and supplied by Dr N. Bateman. All other strains had been bred in our colony for several generations: the fully inbred strains were C57BL, RIII, SM, LG, BSVS, BALB/cf, NZBf, 129f, A2Gf, C3Hf, CBAf and DBA/2f; the partially inbred strains derived from Moredun Institute random-bred mice, and ranging from 6 to 13 generations of sib mating, were MM, EM, LM and VM (referred to as 2M, 3M, 4M and 5M in some previous publications). The colony was free from ectoparasites throughout and the general standard of health high, the only exception being proneness of male MM mice to urinary tract infection with *Proteus mirabilis* and the occurrence of a number of male deaths, of unknown etiology, in several mouse strains in the two experiments 22A-2A and 22A-2G.

The VM stock has been shown (Dickinson, Meikle & Fraser, 1968) to differ from the rest in having prolonged incubation of the ME7 agent, being homozygous for the p7 allele of the gene *sinc*: this allele does not occur in the other stocks.

### (ii) *Origin of 22A and ME7 agents*

*Agent 22A*. Random-bred Moredun Institute mice, C57, RIII, SM, $SS_x$, $LS_x$ and F mice were injected intracerebrally at weaning with a 10 % saline suspension of SSBP/1 scrapie-brain pool at its twenty-first sheep passage, mainly in Cheviot sheep. A clinical disease resembling mouse scrapie developed between 430 and 700 days after injection in at least 3 % of RIII, 11 % of $SS_x$ and 42 % of Moredun random-breds: these are given as minima because the clinical syndrome was insidious and difficult to characterize exclusively and few of the animals were checked histologically at this early stage of the work. A 10 % saline suspension of brain, from one of these Moredun random-bred females killed after 546 days incubation with advanced scrapie symptoms, was used for passage into BSVS, RIII and MM mice. The incidence of clinical scrapie in these second-passage mice was 100 % and diagnosis was confirmed using histological criteria: individual

incubation periods ranged from 308 to 450 days. These mice, which were used as sources of inoculum in a number of the experiments, are referred to as experiment 22A-1A animals.

*Agent ME7.* This was supplied by Dr I. Zlotnik at its second mouse passage in random-bred Moredun mice in which it had been isolated from a natural case of scrapie in a Suffolk sheep by intragastric injection of spleen (Zlotnik & Rennie, 1963). It has subsequently been passaged by intracerebral injection of brain, once in random-bred Moredun mice and then, as sublines within different mouse strains.

### (iii) *Neuropathology*

Histological examination was made of the brains of all mice, irrespective of the reason for killing, so as to confirm the clinical evidence. Although there are detailed differences between the distribution of lesions produced by the two agents, the lesions with both are of the same general type (H. Fraser & A. G. Dickinson, unpublished).

### (iv) *Definition of incubation period*

The criteria are generally those given previously (Dickinson, Meikle & Fraser, 1968). The clinical scoring system has been amplified to accommodate differences in the syndrome between the agents (see 'Results').

*Statistics used.* Incubation periods for subgroups are given as the average ± standard error. This is used in preference to median survival time ($ST_{50}$) because the individual values are approximately symmetrically distributed about the mean on the linear time-scale used, even in the groups with high variance or with large mean. The chosen statistic therefore yields the more efficient information from the data where the incidence of scrapie is 100 %. However, in the high dilutions of titration experiments the incidence is lower and allowance for this complication must be made in interpreting the results, because unaffected animals are not included in the average.

### (v) *Preparation of inocula and injection procedure*

All inocula were prepared from brains of mice, killed during the week following incubation period end-point, removed using full aseptic techniques so as to avoid cross-contamination, and stored in separate bottles at −30 °C. With two exceptions, inoculum was freshly prepared supernatant of a 1 % saline suspension of brain which had been centrifuged at 500 *g* for 10 min: this was administered to 3- to 4-week-old mice as a 0·02 ml dose into the centre of the right-hand cerebral hemisphere using a 26-gauge needle. Inoculum for experiment 35A-2Q differed in the following respects: the original supernatant was frozen in small aliquots, stored for a few weeks at −30 °C, thawed immediately prior to use, centrifuged at 2000 *g* for 15 min and this supernatant used as inoculum; there was a tenfold loss of activity due to these additional procedures. The inocula for titrations differed from the standard in that the supernatant of a 10 % suspension, centrifuged at 2000 *g* for 15 min, was used to make up the various dilutions.

(vi) *Design of experiments*

The designs were intended to minimize any subjective biases in measurement of incubation period, especially any which might operate differentially between different mouse strains injected with the same agent. Exact details differ according to experiment but were generally as follows: separate cage racks were used for the two agents, but apart from this there was only coded information on each cage, to prevent immediate knowledge of what challenge, if any, a particular mouse had received. In titrations mice receiving the same dilution were randomized between cages. Uninjected or saline injected controls, including all mouse strains involved, were present, one to a cage, in up to one quarter of the cages. Where several mouse strains were involved in an experiment these were randomized between cages prior to injection. Mice were observed until they developed scrapie or became senile.

## 3. RESULTS

The incubation periods are measured by scoring the clinical stage of the disease and differences of clinical syndrome were encountered in mice injected with the different agents.

(i) *Clinical syndrome*

Mice injected with 22A agent frequently display chronic progressive ataxia, in contrast with ME7-injected ones of the same stock where the usual syndrome is a progressive lethargy. These alternative courses appear to represent the same phase of the disease, because deaths which can only be attributed to scrapie begin to occur in both cases during this stage. There are differences of detail between strains of mice but the only major complication, which will be evident from the following sections, is that the age at which the clinical stage is reached is very different for the two agents in all stocks other than VM. The very long incubation period of 22A in most stocks is accompanied by a slower progression of the clinical course than for ME7. However, that this difference is due not only to age or length of incubation is demonstrated by VM mice which also have a more chronic course with 22A, despite a somewhat shorter incubation than with ME7.

The effect of the disease on body weight also shows some differences between agents in some stocks. Thus, with 22A agent VM mice show chronic progressive loss of about one-third of the body weight extending over the final quarter of the incubation period. There is no corresponding chronic loss when ME7 is used in the VM stock, apart from a rapid loss during the final week of up to a third of the weight which can occur, and is seen in many strains, in the lethargic phase when there is reduced intake of food and water. C57 mice given ME7 usually show this rapid loss during the final week, but after infection with 22A they have virtually no weight loss up to the end-point.

(ii) *Incubation periods*

In all the experiments, except at the higher dilutions in titrations, the incidence of scrapie was 100 %.

*22A agent: experiments involving several strains of mice and agent from different mouse-passage lines*

*First 22A experiment* (*22A-2A*). Inoculum was derived from two second-passage MM mice, each contributing an equal proportion to the pool. The donor incubation periods were 402 and 408 days in experiment 22A-1A. The incubation periods for the seven mouse strains challenged are given in Table 1.

The most striking feature is the very large range of individual incubation periods—between 100 and 200 days according to mouse strain—and this renders the mean differences between strains statistically non-significant. The VM strain had the smallest variation and the lowest mean incubation period. The shortest individual incubation period was 195 days, occurring in two C57 males.

The high variance was a novel result for us, contrasting with all our previous work where mouse-passaged scrapie at high intracerebral dose had been employed. Another broadly similar experiment (22A-2G) was therefore set up, using agent from a different source animal.

*Second 22A experiment* (*22A-2G*). Inoculum was from a second-passage MM mouse which had a 458-day incubation period in Expt. 22A-1A. The resulting incubation periods are given in Table 1.

The VM stock again has the shortest incubation period, differing significantly from the others in this experiment ($P < 0{\cdot}001$), and the mean is lower and the variance much lower than in the first experiment (22A-2A). The decreased range of incubation period—here about 50 days—applies equally to all the mouse stocks, even though the mean is very much higher, for all stocks except VM, than in the preceding experiment.

Only one mouse, an RIII (335 days incubation), developed scrapie midway in the gap of 148 days between the last VM and the earliest individual in the late group of strains. This gap is virtually the same as the period over which all the cases appeared in the first experiment. Analysis of variance of incubation period, excluding all VM mice and the deviant RIII mouse, shows significant variation among the group of long-incubation period strains ($P$ 0·01–0·001).

In order to check whether the clear distinction of the VM strain from the rest in this second experiment was repeatable, further passages were made.

*22A fourth-passage* (*22A-3E*). Inoculum was derived from a 217-day incubation period VM in 22A-2G. Incubation periods for three strains of mice challenged intracerebrally are given in Table 1. Also mice of these strains were given an intraperitoneal injection (0·1 ml) with the same inoculum and the resulting incubating periods were: VM, $302 \pm 17$ days; C57, $613 \pm 12$ days: LG, $583 \pm 6$ days. Thus, all VM mice developed scrapie, whether challenged intracerebrally or extraneurally, before any C57 or LG mice showed signs of the disease.

*22A fifth-passage* (*22A-4M*). Inoculum was from a VM which had a 194-day incubation period following injection with agent from a VM in Expt. 22A-2A. The results are given in Table 1 for the two mouse stocks injected.

The incubation periods in the C57 mice of both 4th and 5th passage agent are

very similar, as are those for the VM mice, even though the inocula used stem from different third-passage experiments, at which stage one showed high variation of incubation period within stocks and low variation between any stocks (22A-2A), whereas the converse applied for the other (22A-2G).

*ME7 agent: experiments involving several mouse strains and using agent from different sources*

*ME7 third-passage experiment (20A-1A).* These results are abstracted from published data (Dickinson & Mackay, 1964); they are included in Table 1 for ease of cross reference. The dose used was ten times that for most of the experiments reported here: this higher dose shortens incubation by about 7 days in short incubation period stocks and by 20–30 days in the VM stock (see later section). The agent came from a pool of Moredun random-bred mice.

*ME7 fifth-passage experiment (35A-2Q).* The subline of agent came from C57 mice in the above experiment (20A-1A) and had been passaged once in C57 mice before use in this experiment. Many strains of mice were challenged in searching for ones with deviant incubation period: results for six strains in common with other experiments are given in Table 1. The results for six other strains, all with relatively short incubation periods (days), are: NZB, $190 \pm 3$; A2G, $180 \pm 3$; C3H, $172 \pm 0$; 129, $169 \pm 0$; CBA, $178 \pm 3$; DBA, $181 \pm 11$. With the exception of VM, there were no significant differences in incubation period between the other eleven stocks ($P > 0{\cdot}05$).

*ME7 intra-strain passage sublines.* Serial passage of ME7 agent within separate mouse strains has been undertaken in which mice are only challenged with agent from members of their own stock. Nine such sublines, stemming from third-passage material (Expt. 20A-1A) have now been passaged from two to six times in the various strains: the results, averaged over passages, are given in Table 1. The pattern of results is essentially the same as in the other ME7 tests, which proves that the major characteristics of ME7 are independent of the type of donor mice and the differences from 22A are not dependent on the particular donor mouse strains used.

*ME7 fifth-passage experiment (62B-1D).* This subline of agent came from a group of LM mice in Expt. 20A-1A and had been passaged once in VM mice before use in this experiment: inoculum was from a group of these VM mice. The results are given in Table 1 for the two mouse stocks injected.

### (iii) *The response of C57 and VM mice to different concentrations of scrapie agent*

There are two aims in this part of the work: to determine whether both agents reach similar titres in the brain and whether mouse strains which differ in incubation period when given a high dose show a similar difference when given a low dose.

The dose–response curves for the various titrations (Table 2; Fig. 1) are all of the same general shape, showing progressive increases in incubation period as dose decreases. It is clear that the basically different responses of the different

Table 1. *Incubation periods (days) for 22A and ME7 agents injected intracerebrally into weanling mice of different strains*

| Agent | 22A | | | | ME7 | | | |
|---|---|---|---|---|---|---|---|---|
| No. of mouse passages of agent | 3 | 3 | 4 | 5 | 2 | 5 | 4–8 | 5 |
| Concentration of scrapie brain tissue in inoculum (%) | 1 | 1 | 1 | 1 | 10 | ~ 0·1* | 1 | 1 |
| Agent-donor strain | MM | MM | VM | VM | Random-bred | C57 | Same as recipient | VM |
| Experiment no. | 22A–2A | 22A–2G | 22A–3E | 22A–4M | 20A–1A | 35A–2Q | Intrastrain passages | 62B–1D |

| Mouse strain | *n*† | Inc. period‡ | *n* | Inc. period | *n* | Inc. period | *n* | Inc. period | *n* | Inc. period | *n* | Inc. period | *n* | Inc. period | *n* | Inc. period |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VM | 15 | 245 ± 7 | 12 | 226 ± 3 | 19 | 193 ± 3 | 10 | 201 ± 3 | 8 | 280 ± 6 | 6 | 310 ± 6 | 28 | 274 ± 4 | 5 | 333 ± 3 |
| C57BL | 18 | 261 ± 12 | 12 | 442 ± 5 | 6 | 434 ± 6 | 9 | 466 ± 4 | 38 | 158 ± 1 | 7 | 174 ± 1 | 26 | 156 ± 1 | 5 | 171 ± 2 |
| RIII | . | ... | 11§ | 408 ± 8 | . | ... | . | ... | 6 | 146 ± 2 | 5 | 171 ± 2 | 20 | 148 ± 4 | . | ... |
| BSVS | 10 | 263 ± 11 | 11 | 460 ± 4 | . | ... | . | ... | 25 | 158 ± 1 | 5 | 171 ± 1 | 11 | 142 ± 1 | . | ... |
| EM | 5 | 289 ± 32 | . | ... | . | ... | . | ... | . | ... | . | ... | 10 | 187 ± 3 | . | ... |
| MM | 6 | 293 ± 22 | 6 | 464 ± 3 | . | ... | . | ... | 4 | 178 ± 5 | . | ... | 14 | 181 ± 2 | . | ... |
| LM | 14 | 303 ± 16 | 8 | 465 ± 7 | . | ... | . | ... | 4 | 149 ± 6 | 3 | 171 ± 8 | . | ... | . | ... |
| SM | 7 | 307 ± 14 | 8 | 441 ± 10 | . | ... | . | ... | 14 | 155 ± 1 | 6 | 174 ± 1 | 21 | 159 ± 1 | . | ... |
| BALB/c | . | ... | 10 | 460 ± 4 | . | ... | . | ... | . | ... | . | ... | 6 | 164 ± 5 | . | ... |
| LG | . | ... | 10 | 442 ± 4 | 18 | 409 ± 6 | . | ... | 17 | 153 ± 2 | . | ... | 14 | 160 ± 1 | . | ... |

* See text. † Number of mice. ‡ Mean ± standard error. § Excluding highly deviant 335-day incubation period mentioned in text.
... = not done.

Table 2. *Incidence of scrapie* (%) *and incubation period* (*days*) *for serial ten-fold dilutions of ME7 or 22A scrapie brain injected intracerebrally*

| Agent ... ... | ME7 | | | | | | | | 22A | |
|---|---|---|---|---|---|---|---|---|---|---|
| Assay stock ... | C57BL | | | | | | VM | | VM | |
| No. of mouse passages ... | 3 | | 4 | | 4 | | 4 | | 4 | |
| Experiment no. ... | 35A-1A | | 35A-2D | | 35A-2Q | | 35A-2M | | 35A-2Q | | 22A-3J | |
| Dilution | Inc.* period | % incidence | Inc. period | % incidence | Inc. period | % incidence | Inc. period | % incidence | Inc. period | % incidence | Inc. period | % incidence |
| $10^{-1}$ | 154 ± 3 | 100 | ... | ... | ... | ... | 294 ± 4 | 100 | ... | ... | 190 ± 4 | 100 |
| $10^{-2}$ | 161 ± 1 | 100 | 164 ± 1 | 100 | 174 ± 1 | 100 | 312 ± 6 | 100 | 310 ± 6 | 100 | 198 ± 2 | 100 |
| $10^{-3}$ | 168 ± 1 | 100 | 173 ± 1 | 100 | ... | ... | 345 ± 4 | 100 | ... | ... | 220 ± 7 | 100 |
| $10^{-4}$ | 176 ± 2 | 100 | 189 ± 4 | 100 | 198 ± 7 | 100 | 395 ± 14 | 100 | 414 ± 24 | 100 | 233 ± 5 | 100 |
| $10^{-5}$ | 184 ± 1 | 100 | 221 ± 6 | 100 | 249 ± 16 | 60 | 410 ± 7 | 100 | ... | ... | 274 ± 14 | 60 |
| $10^{-6}$ | 231 ± 7 | 100 | 274 ± 31 | 44 | 270 ± 24 | 40 | > 638† | 25 | — | 0 | 282‡ | 20 |
| $10^{-7}$ | 262 ± 12 | 50 | 364 ± 92 | 18 | — | 0 | — | 0 | ... | ... | — | 0 |
| $10^{-8}$ | 328 ± 21 | 27 | — | 0 | — | 0 | ... | ... | ... | ... | ... | ... |
| Titre (Reed–Muench) | −7·2 | | −6·1 | | −5·9 | | −5·7 | | — | | −5·3 | |

* Mean ± standard error. † Only one case; killed because of mammary tumour but showing early scrapie brain lesions. ‡ Only one case.
... = not done. — = not applicable.

mouse genotypes to the two agents cannot be explained solely in terms of differences of dose. There has been no attempt yet to titrate 22A in C57 mice because of the practical difficulty that, if the response curve is similar to the others, the incubation periods for the higher dilutions are liable to approach the normal life-span, which would make it difficult to be certain that even higher dilutions produced negative results.

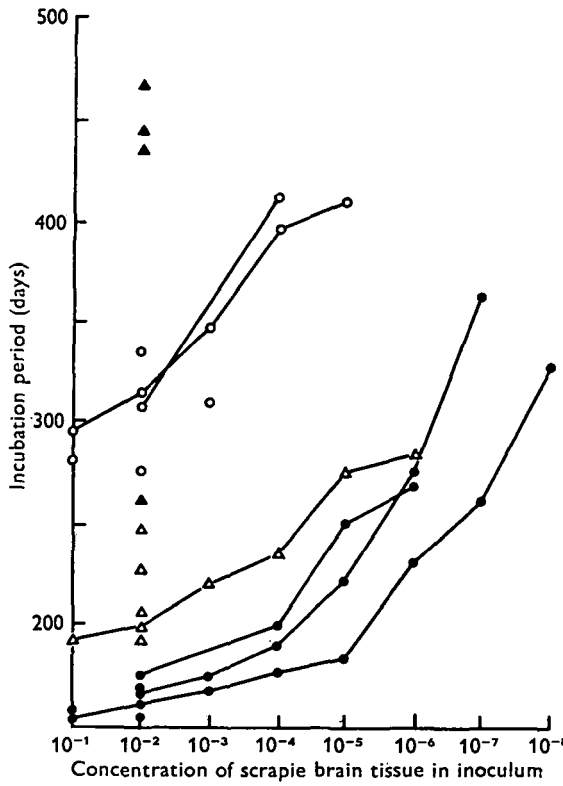


Fig. 1. Incubation periods for C57 and VM mice injected intracerebrally with various doses of ME7 or 22A scrapie brain material. Points for each serial dilution experiment are joined by lines. ○, ME7 in VM; △, 22A in VM; ●, ME7 in C57; ▲, 22A in C57.

The survivors in the titrations were observed till senile (up to 24 months old) and their negative status confirmed histologically: details of the experiments were as follows:

*ME7 titration 1* (*35A-1A*). Inoculum was from a C57 mouse in Expt. 20A-1A and 12 to 15 C57 mice of each sex were injected with each serial log dilution. The results for the two sexes do not differ significantly ($P > 0{\cdot}1$).

*ME7 titration 2* (*35A-2D*). Pooled agent from mice in the first titration (35A-1A) was used to inoculate groups of 10 to 12 C57 mice at each dilution.

*ME7 titration 3* (*35A-2Q*). The inoculum, which was the same as for the ME7

fifth-passage experiment (35A-2Q) was injected into both C57 and VM mice—five to seven of each strain were used for each dilution.

*ME7 titration 4 (35A-2M)*. Inoculum, as for the second titration (35A-2D) but prepared separately, was injected into six to eight VM mice for each dilution.

*22A titration 1 (22A-3J)*. Inoculum was from the same source as 22A-3E given previously; five VM mice were inoculated at each dilution.

## 4. DISCUSSION

The different ME7 titrations demonstrate the high degree of repeatability of the relationship between incubation period and the concentration of this agent, within a given genotype of assay animal. If the ME7 results (Table 2) are corrected to a common incubation period basis to allow for differences in the starting concentration of scrapie agent, the dose–response curves are remarkably similar to one another, within one strain of mouse. The published results for a scrapie agent with a different origin are also broadly similar to our own (Hunter, Millson & Chandler, 1963).

Table 3. *The reversal of relative incubation periods (days) in C57 and VM mice according to the agent used (values based on results in Table 1 but excluding 22A-2A)*

| | Mouse strain | |
|---|---|---|
| Agent | C57 | VM |
| ME7 | 165 | 299 |
| 22A | 443 | 205 |

If there had been diversity in the ME7 dose–response relationships it might have been possible to regard the 22A agent as no more than an extreme variant differing in some quantitative property from ME7, though this difference could not have been in brain titre as this is of the same order for both agents. In C57 mice, with the exception of the first 22A experiment (22A-2A) which is discussed later, the incubation period following a high dose of 22A is longer and much less variable than for ones receiving the minimal dose of ME7. This illustrates the magnitude of the difference, but it is suggested that these agents differ more fundamentally than in mere quantitative properties. The basis for this suggestion is the reversal of relative incubation period of the two agents in the C57 and VM stocks as summarized in Table 3.

It is surprising that, of the large number of mouse stocks tested, the VM strain was the only one distinct from the remainder with both ME7 and 22A. The incubation of ME7 has been shown to be almost entirely determined by one gene, *sinc*, of which VM mice have the p7 alleles and the remainder have alleles indistinguishable, at present, from the s7 allele identified in the RIII strain (Dickinson, Meikle & Fraser, 1968). It is possible that the same gene determines incubation of the 22A agent, in which case each allele would have to have opposite effects with each of these two agents; this is currently being tested.

It is also possible that other genes may play very minor roles in controlling incubation period, and the evidence indicates that their effect may be the same with both 22A and ME7. Of the short-ME7 group of strains, RIII is usually one of the shortest: this is shown in the present data and in a variety of our unpublished results. In contrast, MM is usually one of the longest of this group. Other strains tend to recur in intermediate positions over a range of experiments. The results for the first 22A experiment (22A-2A) are too variable to draw this type of conclusion, but it is apparent from the second experiment (22A-2G) that the same ranking as for ME7 holds, even though the average incubation periods are almost three times as long.

It will be very difficult to titrate 22A agent, with the properties shown at 4th and 5th passage, in any mice other than the VM stock because the incubation period is likely to exceed the life span at the higher dilutions. The same limitation applies to investigations using extraneural routes unless very high doses are used. The results given for such work in C57 and LG mice show that ones injected as weanlings were nearly 2 years old when scrapie developed, at which age some mice of these strains show signs of senility. This finding raises a more general problem, namely that infection with 22A can be so prolonged that clinical disease is not seen during the animal's lifetime. This is fundamentally distinct from true latency, such as in human herpes infection, because the scrapie agent is recoverable for the larger part of the incubation period; the disease is progressing throughout this time and therefore displays characteristics in common with slow virus infections (Sigurdsson, 1954) rather than with latency, in the context in which that term is ordinarily used. The finding of a scrapie agent with these properties obviously suggests the possibility that another variant with even more extreme properties might exist which never manifests as a clinical disease in some genotypes. Indeed, this possibility is being considered for the Cheviot sheep we have bred which are genetically 'resistant' to challenge with SSBP/1 scrapie agent (Dickinson, Stamp, Renwick & Rennie, 1968).

Quite apart from the problem which slow infection of this type might present for attempts at eradication of scrapie in sheep, the present findings are a clear indication that the hope of finding a 'scrapie-resistant sheep genotype' represents an oversimplified view of the problem. Judging from the findings in mice, sheep of different genotypes are quite likely to be found to vary in their 'resistance', as defined operationally, according to the particularly scrapie agents which they encounter: some evidence in support of this has been found (Dickinson & Stamp, 1969). In practice, eradication may be to some extent simpler than these comments suggest. This is because the predominant role of maternal transmission in sheep (Dickinson, Young, Stamp & Renwick, 1965) will tend to limit the range of agents to which any particular genotype is exposed, but this will only apply if postnatal contagion is infrequent.

Finally we turn to the question of the possible nature of the agent differences which have been shown. A disease organism can be more virulent, in the widest sense, for one strain of host than another for two reasons, which also apply to the

case where two strains of the organism differ in virulence for one genotype of host. Either the micro-organism can be specially adapted to one host phenotype, as could arise from the incorporation of host-specific proteins into its make-up, or there may be genetical properties of the micro-organism which elicit diverse responses from hosts of different genotypes, or both causes could be involved. The possibility that the major differences in the present data between the 22A and ME7 agents could be entirely accounted for by some host component in the structure of the scrapie organism, is ruled out by the persistence of the basic difference on serial passage of each agent in the VM strain. If a host component is involved it must be accompanied by other more inherent differences, but this does not automatically imply the occurrence of genetical variation in the scrapie organism for the following reasons.

In the present case, where a crude inoculum is used instead of a pure preparation of the pathogen, additional possibilities may lead to results which differ between inocula from different sources. Donor tissues present in the inoculum may interfere in the disease process and any such differences between inocula would have to be taken into account in interpreting the results. Such donor tissue effects could come either from genetic differences between donor and recipient or from organ-specific effects within a genotype where different types of tissue are used as inoculum—an example of the latter has been shown to modify scrapie response (Fraser & Dickinson, 1967). This phenomenon cannot be invoked to explain the agent strain differences in the current data because brain is the only source of inoculum and major differences between agents have been shown to persist when using the same genotype of donor and host for both 22A and ME7 agents.

A second possibility, where a crude inoculum is used, is that another micro-organism may be present which is biologically associated with the scrapie agent or which influences the disease process. If this were so, apparent agent differences could arise from variation in the associated organism or its presence or absence. The only evidence in the current results which might lend support to this possibility is the atypically high variance of incubation period, within mouse strains, experienced in the first 22A experiment (22A-2A). If infection depended on a 'double-hit' type of model, where scrapie and another micro-organism were simultaneously necessary and one or both of them was rarely present at the cellular site of infection, then variance of incubation period would be high. This would contrast with the low variance which would result either from an abundance of both components or where only one was needed. However, preliminary results from prolonged, intense ultraviolet irradiation of the two agents, indicates that their distinctive properties are retained. If this is so, the possibility of an associated micro-organism can probably be ruled out because it is unlikely that it would share the resistance to u.v. displayed by scrapie.

This leaves a mixture of scrapie agents as a more likely explanation of the high variance results. An inoculum could contain a very disproportionate mixture of two or more scrapie agents of differing virulence as measured by incubation period. If very few infectious units were present of the more virulent agents then

incubation period could be very variable, depending on the relative doses of the different agents which each individual received.

Although the present results are not sufficient to discount completely the other possibilities, the balance of evidence indicates that the major difference between the two agents is of a genetical nature in the scrapie organism.

## SUMMARY

Two mouse-adapted scrapie agents of different sheep origin were compared. The titre, reached in the brains of mice in the terminal stage of scrapie, is of the same order for both agents. There is a threefold difference between the incubation periods of the two agents in some mouse strains, of which C57 is one, and in this strain incubation of the 22A agent, given as a large dose by a peripheral route, occupies almost the whole life-span.

The most fundamental difference between the agents concerns the reversal of the ranking of incubation periods, typically in the VM and C57 mouse strains: incubation of ME7 in VM takes almost twice as long as in C57, whereas most sublines of 22A take half as long in VM as in C57. The implications of this type of host-genotype, agent-strain interaction are discussed in terms of the possible nature of agent differences, the possibility of latent infection and the consequences for scrapie eradication programmes.

## REFERENCES

Dickinson, A. G. & Mackay, J. K. M. (1964). Genetical control of the incubation period in mice of the neurological disease, scrapie. *Heredity* **19**, 279–288.

Dickinson, A. G., Meikle, V. M. H. & Fraser, H. (1968). Identification of a gene which controls the incubation period of some strains of scrapie agent in mice. *J. comp. Path.* **78**, 293–299.

Dickinson, A. G. & Stamp, J. T. (1969). Experimental scrapie in Cheviot and Suffolk sheep. *J. comp. Path.* **79**, 23–26.

Dickinson, A. G., Stamp, J. T., Renwick, C. C. & Rennie, J. C. (1968). Some factors controlling the incidence of scrapie in Cheviot sheep injected with a Cheviot-passaged scrapie agent. *J. comp. Path.* **78**, 313–321.

Dickinson, A. G., Young, G. B., Stamp, J. T. & Renwick, C. C. (1965). An analysis of natural scrapie in Suffolk sheep. *Heredity* **20**, 485–503.

Fraser, H. & Dickinson, A. G. (1967). Distribution of experimentally induced scrapie lesions in the brain. *Nature, Lond.* **216**, 1310–1311.

Hunter, G. D., Millson, G. C. & Chandler, R. L. (1963). Observations on the comparative infectivity of cellular fractions derived from homogenates of mouse-scrapie brain. *Res. vet. Sci.* **4**, 543–549.

Pattison, I. H. (1966). The relative susceptibility of sheep, goats and mice to two types of the goat scrapie agent. *Res. vet. Sci.* **7**, 207–212.

Pattison, I. H. & Jones, K. M. (1968). Modification of a strain of mouse-adapted scrapie by passage through rats. *Res. vet. Sci.* **9**, 408–410.

Sigurdsson, B. (1954). Rida, a chronic encephalitis of sheep with general remarks on infections which develop slowly and some of their special characteristics. *Brit. vet. J.* **110**, 341–354.

Stamp, J. T. (1967). Scrapie and its wider implications. *Br. med. Bull.* **23**, 133–137.

Zlotnik, I. & Rennie, J. C. (1963). Further observations on the experimental transmission of scrapie from sheep and goats to laboratory mice. *J. comp. Path.* **73**, 150–162.