Scrapie-induced Alterations in Glucose Tolerance in Mice

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(Accepted 19 December)

SUMMARY

Certain scrapie strains cause obesity in several strains of mice. The potential association between obesity and altered glucose tolerance was assessed by monitoring body weight and glucose tolerance throughout the incubation period in scrapie strain–mouse strain combinations that do and do not produce obesity. Virtually all obese mice showed reduced glucose tolerance as shown by significantly higher blood glucose levels 2 h after a glucose overload. Mice injected with a scrapie strain that did not cause obesity showed normal tolerance. The scrapie infectivity titre of the pancreas of obese mice clinically affected with scrapie was very low. Adrenalectomy prevented both the increase in weight and aberrant glucose tolerance but had no other effect on the course of the disease. Following increasing dilution of the inoculum, the increase in body weight and the development of aberrant glucose tolerance reached an end-point that was similar to that of scrapie infectivity. The system described provides an inducible model of obesity with altered glucose tolerance.

INTRODUCTION

Of the group of human and animal diseases caused by unconventional agents, most experimental work has been done with scrapie, which is a natural disease of sheep and goats (Fraser, 1983; Carp et al., 1985a). The scrapie agent has been passaged in small laboratory animals, such as mice and hamsters, in which the course of the disease is controlled by certain genetic characteristics of both the host and the agent (Dickinson & Fraser, 1979; Fraser, 1979). Although the nature of this group of infectious agents remains unknown, the precision of the interaction between agent and host has permitted insights into the pathogenesis, genetics and characteristics of the disease (Dickinson & Fraser, 1979; Carp et al., 1985b).

The initial signs used to diagnose clinical scrapie disease include incoordination, weakness and in some cases a brief period of hyperexcitability. Subsequent signs include a loss of weight, progressive loss of motor capability and activity, a hunched stance, paresis and paralysis followed by death. Changes in the host that do not involve direct action of the agent on motor functions have received little attention in studies on scrapie or the other unconventional diseases. During scrapie infection, studies have been made of food and water intake, increases in body weight prior to the onset of motor changes (Outram, 1972; Carp et al., 1984) and behavioural changes measured in terms of emergence times (Heitzman & Corp, 1968) and defaecation scores (Savage & Field, 1965). Changes were also seen in open-field and Y-maze exploration scores (McFarland et al., 1980) and in apomorphine-induced circling behaviour after unilateral stereotaxic injection of the nigrostriatal system (Gorde et al., 1982). Our recent studies which focused on the obesity seen before motor changes in certain scrapie strain–mouse strain combinations (Carp et al., 1984; Carp & Callahan, 1984) have established the importance of the genetic characteristics of host and agent, the role of the hypothalamus (Kim et al., 1987a) and more recently the function of the adrenal gland in the weight increase (Kim et al., 1988).

The increase in weight of animals in these studies was caused by an accumulation of fat and not an increased growth rate (Carp et al., 1984). This suggested that alterations in glucose
provided by Dr Alan Dickinson (AFRC and MRC Neuropathogenesis Unit, Edinburgh, U.K.) and has been maintained in our animal colony as an inbred strain by random brother-sister matings for the past 10 years. All with controlled temperature, humidity and light cycle (12 h on, 12 h off). The IM/Dk mouse strain was kindly metabolism might accompany obesity and led to the series of studies reported in this paper, in which we examined glucose tolerance changes and their relation to increased weight gain.

**METHODS**

**Mice.** Female weanling C57BL/6J and SJL/J mice (Jackson Laboratories) were housed in animal colony rooms with controlled temperature, humidity and light cycle (12 h on, 12 h off). The IM/Dk mouse strain was kindly provided by Dr Alan Dickinson (AFRC and MRC Neuropathogenesis Unit, Edinburgh, U.K.) and has been maintained in our animal colony as an inbred strain by random brother–sister matings for the past 10 years. All mice were given rodent food and water or 0-9% NaCl (for mice that were adrenalectomized) freely. Mice were ear-punched for identification and weighed just before injection. Subsequently, mice were weighed in the early afternoon of the same day of the week, with the schedule being either every week or every other week for the short incubation scrapie models and either every other week or once per month for long incubation models.

**Scrapie strains and isolates.** The ME7, 22L and 22A scrapie strains were also obtained from Dr Alan Dickinson. A recently established line, termed C602, was derived from a Suffolk sheep affected by scrapie. The line was used for four or five mouse-to-mouse passages in C57BL mice. For all scrapie strains and lines the material to be used for injection was prepared by passage using intracerebral (i.c.) injection of 0.03 ml of 1% (w/v) brain homogenates from mice affected by scrapie. C57BL mice were used for passaging strains ME7 and 22L and line C602. For the 22A scrapie strain, the IM mouse strain was used for passaging.

**Preparation of material for injection.** Brain material for injection was homogenized in phosphate-buffered saline (PBS). Homogenates (10% w/v) were prepared by 20 strokes of a hand-operated Ten-Broeck homogenizer. Normal brain homogenate was prepared from C57BL mice injected with normal mouse brain (NMB) homogenate. Homogenates (both normal and scrapie-affected) were stored at −70°C, thawed and then diluted to an appropriate concentration in PBS just before injection.

**Injection.** Stereotaxic microinjections were carried out under general anesthesia [sodium pentobarbital, 70 mg/kg intraperitoneal (i.p.)] after mounting the mice on a stereotaxic instrument (Stoelting). The choice of the right side for injection was made arbitrarily, since no cerebral dominance has been established in small rodents. The coordinates used for the hypothalamus were A-1.8, L+0.5, H+5.2 (Slotnik & Leonard, 1975). Using a 30-gauge stainless steel needle, 5 μl of a 1% brain homogenate was injected. In preliminary experiments, carbon particles were included in the inoculum and after stereotaxic injection these particles were shown to be localized in the area designated by the coordinates. For routine i.c. injections, 0.03 ml of the appropriate dilution was used, whereas for the i.p. route, 0.2 ml was injected.

**Analysis of clinical symptoms.** Mice were monitored weekly for typical motor signs of scrapie starting at 70 days post-injection (p.i.) for short incubation models and starting at 175 days p.i. for long incubation period models. Clinical disease was assessed by observing the activity levels of mice and their competence on an apparatus consisting of a series of parallel bars (3 mm in diameter) placed 7 mm apart (Carp et al., 1984). With the agent–mouse strain combinations used, the initial clinical findings are a reduction in activity and in the ability of the mice to traverse the parallel bars. The incubation period was the point at which mice had shown typical motor signs for the third consecutive week. Incubation periods are expressed as mean ± standard error (s.E.).

**Adrenalectomy.** For adrenalectomy, mice were anesthetized with sodium pentobarbital (70 mg/kg) and then adrenalectomized by the lumbar approach (Debons et al., 1982). Mock-operated mice were exposed to the same operative procedure, except for removal of the glands. Mice were adrenalectomized and mock-operated 2 to 3 weeks before injection.

**Glucose tolerance test.** Samples (50 to 200 μl) were obtained via retro-orbital (right orbit) bleeding at least 1 h before glucose loading. Heparinized micro-hematocrit tubes (Monoject) were used. The glucose loading was a standard dose of 2 mg/g body weight injected i.p. A post-glucose blood sample (from the left orbit) was obtained 2 h after overload. Mice were bled repeatedly with intervals as short as 13 days; no difficulties were encountered because of the multiple bleedings. In initial experiments, plasma glucose levels were determined by a colorimetric enzymic method (Sigma). In subsequent experiments, whole blood glucose was measured photometrically using the Glucoscan 2000 Blood Glucose Meter (Lifescan).

**RESULTS**

**Glucose tolerance test (GTT) analyses in short incubation models injected i.p.**

The ME7 strain causes a weight increase in SJL mice after injection either i.c. or stereotaxically into the hypothalamus (Carp et al., 1984; Carp & Callahan, 1984, Kim et al., 1987a). In the present study, SJL mice were injected i.p. with ME7 or NMB and body weights were monitored. ME7-injected mice showed significantly increased weight at 196 and 210 days p.i. (Table 1). The incubation period for this combination was 223 ± 1 days. After establishing
Scrapie alterations in glucose tolerance

Table 1. The effect of ME7 on the weight of SJL mice after i.p. injection

<table>
<thead>
<tr>
<th>Days p.i.</th>
<th>NMB</th>
<th>ME7</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>26.8 ± 1</td>
<td>27.8 ± 1</td>
</tr>
<tr>
<td>168</td>
<td>27.3 ± 1</td>
<td>29.5 ± 1</td>
</tr>
<tr>
<td>183</td>
<td>27.7 ± 1</td>
<td>30.2 ± 1</td>
</tr>
<tr>
<td>196</td>
<td>28.1 ± 1</td>
<td>31.4 ± 1†</td>
</tr>
<tr>
<td>210</td>
<td>28.2 ± 1</td>
<td>32.7 ± 1‡</td>
</tr>
</tbody>
</table>

* Weight ± s.e. where n is 11 to 18.
† Significant difference from NMB: P < 0.05.
‡ Significant difference from NMB: P < 0.01.

Table 2. Weight and GTT values of SJL mice injected with ME7 by the i.p. route

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mouse</th>
<th>Days p.i.</th>
<th>Weight (g)</th>
<th>Pre-overload</th>
<th>Post-overload</th>
<th>Scrapie-positive (day p.i.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>152</td>
<td>39.3</td>
<td>182</td>
<td>302*</td>
<td>182</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>152</td>
<td>44.8</td>
<td>197</td>
<td>339*</td>
<td>189</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>152</td>
<td>30.6</td>
<td>171</td>
<td>268</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>152</td>
<td>34.2</td>
<td>180</td>
<td>211</td>
<td>201</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>152</td>
<td>39.7</td>
<td>216</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>197</td>
<td>71.9</td>
<td>197</td>
<td>288*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>205</td>
<td>71.7</td>
<td>182</td>
<td>375*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>210</td>
<td>71.0</td>
<td>130</td>
<td>313*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>216</td>
<td>60.0</td>
<td>94</td>
<td>621*</td>
<td>187</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>223</td>
<td>49.1</td>
<td>47</td>
<td>636*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>152</td>
<td>44.8</td>
<td>197</td>
<td>339*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>183</td>
<td>50.3</td>
<td>192</td>
<td>225</td>
<td>189</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>205</td>
<td>30.4</td>
<td>80</td>
<td>600*</td>
<td></td>
</tr>
</tbody>
</table>

* Values at least 3 S.D. above normal [average values for 39 SJL mice injected with NMB were 154 ± 25 (mean ± S.D.) and 173 ± 35 (mean ± S.D.) for the pre- and post-overload samples, respectively].

that SJL mice injected i.p. with ME7 show an increase in body weight, glucose tolerance was assessed in five mice before the onset of typical motor signs (Table 2, experiment 1). Glucose values after loading were all high and for two mice the values exceeded 3 S.D. above normal. Most SJL mice injected i.p., i.c. or stereotaxically in the hypothalamus with ME7 attained body weights between 28 and 40 g. However, a number of mice became enormous; the glucose values and weight for two of the i.p. injected mice during a series of repeated tests are shown in Table 2. The pre-glucose values for mouse 6 changed from slightly hyperglycaemic shortly after signs of incoordination began to markedly hypoglycaemic as the mouse lost weight during the progression of clinical disease. The post-overload values increased markedly over the same time period. Mouse 2 showed very high pre-overload values the first two times it was tested and markedly aberrant post-overload values at the first and third tests. Two mice injected i.c. with ME7 that attained body weights of 82.4 and 67.6 g were tested sequentially and showed very high post-overload glucose values and a progressive decrease in pre-overload values (data not shown).

GTT analysis in a short incubation model using mice injected stereotaxically in the hypothalamus

Previous studies showed that the body weight of SJL mice increased after either i.c. or stereotaxic injection with the 22L scrapie strain (Carp et al., 1984; Kim et al., 1987a). These findings led to questions concerning a possible relationship between increased weight and
altered glucose metabolism. In the present study, SJL mice were injected stereotaxically in the hypothalamus with 22L or with NMB. Glucose tolerance and weight were assessed on one set (normal and 22L) at 50, 64, 112 and 128 days and on another set at 79, 93, 106 and 119 days (Fig. 1). Five or six scrapie-injected mice and five or six controls were used in each group. There were significant ($P < 0.02$) differences in weight between the scrapie-infected and normal groups at 93, 106, 112 and 119 days p.i. and significant ($P < 0.01$) differences in glucose tolerance at 106, 112 and 119 days p.i. The difference in GTT values at 64 days p.i. was at the 0.05 level of significance. The scrapie incubation period was 117 days so that the final time point (day 128) was approximately 3 weeks after the initial signs of clinical scrapie. At this point some 22L-injected mice had begun to lose weight whereas others were still gaining weight; this led to an increase in the variance of the mean weight value. The post-overload glucose values also show a marked increase in variance which was probably related to the variation observed in body weight.

Scrapie infectivity in the pancreas

An assessment of the infectivity level in the pancreas of SJL mice injected by the hypothalamic route with ME7 showed an extremely low titre. The sample was taken after the
Scrapie alterations in glucose tolerance

Fig. 3. Weight (a) and glucose level 2 h after glucose overload (b) of C57BL mice injected i.c. with NMB (hatched), the 22A scrapie strain (unshaded) or the C602 line (shaded). The inocula were 1% brain homogenates. Data points are averages of the results from eight to 12 mice for the C602 and NMB groups and three to 12 mice for the 22A group. Bars indicate s.e. Days p.i. are not plotted to scale.

onset of motor signs of disease and the titre was equal to or less than $2.6 \times 10^2$ LD$_{50}$/g which is between $10^2$- and $10^6$- fold less than that seen in brain. Similar low infectivity titres for pancreas were obtained in a second experiment in which ME7 had been injected by the i.c. route.

Analysis of obesity and GTT in long incubation models injected i.c.

During a series of mouse-to-mouse passages of brain homogenates prepared from five scrapie-affected Suffolk sheep, some of the lines caused marked increases in weight before the onset of motor signs of disease. In general, incubation periods became shorter during repeated passages, but for a number of lines incubation periods remained in the range of 285 to 360 days (after injection with a 1% brain homogenate) even by the fifth passage. Sequential weight data for C602, one of these lines, are shown for the fifth mouse passage in Fig. 2. In this experiment C57BL mice were injected i.c. with either a 5% homogenate of NMB or C602 or a 0.01% homogenate of C602. Compared to the NMB group, mice injected with the 5% homogenate of C602 showed significantly higher body weight at 154 days p.i. and the significant difference persisted until the end of the clinical phase. The incubation period was 286 ± 8 days. For mice injected with 0.01% homogenate of C602, the weight increase was significant from 280 days p.i. onward, and the incubation period was 364 ± 4 days. The peak weights reached by groups of mice injected with 5% and 0.01% homogenates were 55.3 and 58.0 g, respectively. These values were almost twice those of control mice, for which average weights were between 30 and 35 g during this period.

The next question was whether the mice that became obese in the long incubation model also developed aberrant GTT results. C57BL mice were injected by the i.c. route with a 1% homogenate of the fifth mouse-to-mouse passage of C602 (Fig. 3). Additional C57BL mice were injected with the 22A scrapie strain or with NMB homogenate, each as a 1% homogenate. Mice were monitored periodically for body weight and glucose tolerance. Values for weight were significantly different between the C602 and NMB groups at 254, 284 and 292 days p.i. with $P$ values of < 0.001, < 0.001 and < 0.01, respectively (Fig. 3a). The post-overload glucose values for the C602-injected group were significantly higher than those for NMB injected mice at 254, 277, 284 and 292 days p.i., with $P$ values of < 0.02, < 0.01, < 0.001 and < 0.01, respectively (Fig. 3b). The incubation period for the C602 isolate (1% homogenate) was 284 ± 5 days. It has been reported that the 22A scrapie strain causes a reduction in body weight before the onset of motor changes (Outram, 1972). In the present study, the weight of 22A-injected mice was significantly lower than that of controls at 254, 277 and 292 days p.i. with an incubation period of 368 ± 0 days. The post-overload glucose values of 22A-injected mice tended to be lower than controls but the difference was significant only at 292 days p.i. ($P < 0.05$).
Table 3. Weight and post-glucose overload values of C57BL mice injected i.c. with high dilutions of homogenate of the fifth passage of the C602 line

<table>
<thead>
<tr>
<th>Scrapie designation</th>
<th>Concentration of homogenate (%)</th>
<th>Weight (g ± S.E.)</th>
<th>Glucose (mg/dl ± S.E.)</th>
<th>Weight (g ± S.E.)</th>
<th>Glucose (mg/dl ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (NMB)</td>
<td>1</td>
<td>38.9 ± 1*</td>
<td>166 ± 9*</td>
<td>38.9 ± 1*</td>
<td>166 ± 9*</td>
</tr>
<tr>
<td>Positive†</td>
<td>0.001 to 0.00001</td>
<td>45.6 ± 3</td>
<td>214 ± 31</td>
<td>44.2 ± 3</td>
<td>216 ± 44</td>
</tr>
<tr>
<td>Negative‡</td>
<td>0.0001 to 0.000001</td>
<td>32.9 ± 3</td>
<td>147 ± 18</td>
<td>39.6 ± 4</td>
<td>145 ± 12</td>
</tr>
</tbody>
</table>

* Values were pooled from samples taken at 309, 326, 374 and 397 days p.i.
† Mice were positive for clinical scrapie at the time of testing or became positive within 4 months.
‡ Mice were negative for clinical scrapie at the time of testing and remained negative throughout the rest of the experiment.

Correlation between infectivity and the induction of obesity and aberrant GTT

All previous studies with the long incubation model had been done with comparatively concentrated inoculum (5% to 0.01%) and the question arose concerning the capacity of more dilute inocula to induce obesity and altered GTT. In an experiment in which C602 was diluted in order to determine its infectivity end-point we assessed both weight and GTT results at various dilutions of the C602 inoculum. In the final three dilutions (representing 0.001, 0.0001 and 0.00001% concentrations of the original homogenate), 19 animals developed scrapie and 13 did not. The 19 mice listed as scrapie-positive either had typical motor signs of scrapie at the time they were tested (day 374 or day 393) or developed scrapie within the next 4 months. As shown in Table 3 those mice at the end-point which developed clinical scrapie had higher total body weight and showed aberrant GTT results compared to mice that did not develop scrapie.

The effect of adrenalectomy on the induction of obesity and aberrant GTT in a long incubation model of scrapie

In a previous study we showed that adrenalectomy prevented the increase in body weight in both SJL mice injected i.c. with ME7 and in C57BL mice injected in the hypothalamus with ME7 (Kim et al., 1988). In the present study, adrenalectomy prevented the weight increase induced by line C602 (Fig. 4a). Mock-operated mice injected with the C602 homogenate showed a significant weight increase compared to mock-operated mice injected with NMB from day 229 on and a significant increase in weight compared to the C602-injected, adrenalectomized mice from day 196 to the end of the experiment. Blood glucose values 2 h after a glucose overload were significantly greater in the C602 mock-operated group compared to the NMB group only at the last two time points (Fig. 4b). Post-glucose overload values for adrenalectomized mice injected with the C602 line were significantly lower than the mock-operated group injected with C602 at all time points from day 164 to day 257. The final samples (day 285) were taken when some of the C602-injected, adrenalectomized mice were showing motor signs of scrapie disease and there was a rise in the average post-overload glucose value. There was also marked variability in the GTT values as shown by the very large S.E. value. The incubation periods for the mock-operated and adrenalectomized mice were 284 ± 2 and 281 ± 2 days, respectively.

DISCUSSION

The current studies describe a previously unreported capacity of certain scrapie strains to induce aberrant glucose tolerance. A number of findings suggest a general association between obesity and glucose intolerance. The change in GTT results and obesity were seen before the onset of motor changes after i.p. and non-stereotaxic i.c. injection and after stereotaxic injection in the hypothalamus; both changes were seen in both short and long incubation period models. In a titration assay for infectivity, there was close agreement between the endpoints of
Infectivity, obesity and altered GTT results (Table 3). In mice that did not become obese, such as C57BL mice injected with 22L or 139A (data not shown) or with 22A (Fig. 3), glucose tolerance was not impaired. Finally, adrenalectomy prevented both the increase in body weight and hyperglycaemia (Fig. 4). In other mouse model systems, obesity and diabetes can occur together or each can occur separately (Coleman, 1978; Craighead, 1981).

In previous studies, the role of genetic control of obesity was emphasized (Carp et al., 1984; Kim et al., 1987a). The linkage between obesity and altered GTT observed in the present study would mean that the latter is also under genetic control. Direct evidence of the influence of scrapie agent genetics on glucose tolerance is shown by comparing the effects of 22A and C602 in C57BL mice (Fig. 3).

The remarkable obesity associated with the long incubation C602 scrapie line in which infected animals attain weights nearly twice that of NMB-injected controls provides a model system which may prove useful for the study of secondary effects of obesity and glucose intolerance. Infected mice maintain their increased weight for as long as 17 weeks and exhibit glucose intolerance for at least 5 weeks before the onset of the typical motor changes associated with scrapie. The extent and duration of these changes could lead to a variety of related effects such as peripheral neuropathy, kidney damage, aberrant cholesterol metabolism and circulatory problems (Keys et al., 1972; Brown & Asbury, 1984).

The hypothalamus plays a role in scrapie-induced obesity in mice (Kim et al., 1987a, 1988). The effect is probably mediated through the hypothalamic–pituitary–adrenal axis. If we assume that aberrant glucose tolerance is secondary to obesity, then the GTT alteration would also be the result of changes in the hypothalamic–pituitary–adrenal axis. The fact that the level of scrapie infectivity is extremely low in the pancreas of clinically affected mice showing aberrant GTT results suggests, but does not prove, that a direct effect of scrapie agent on the pancreas is

Fig. 4. Weight (a) and post-overload glucose values (b) in C57BL mice injected i.c. with either the C602 line or NMB. Before injection (2 to 3 weeks), mice were either adrenalectomized or mock-operated. The four groups were: mock-operated C602 (△); mock-operated NMB (△); adrenalectomized C602 (○); adrenalectomized NMB (●). Bars show s.e.
unlikely. For example, it is possible that abortive infection of pancreatic cells led to their dysfunction and the resultant GTT changes or that the scrapie titre had been high at some point during the preclinical phase of disease at which time the pertinent pancreatic cells had been permanently damaged. In a previous study that demonstrated probable neuroendocrine involvement in experimental scrapie, histopathological changes in ovaries occurred in the absence of high scrapie titres in the affected organ (Sturman, 1972).

The fact that some scrapie strain–mouse strain combinations show obesity and altered GTT results whereas others do not can be explained on the basis of scrapie strain-specific targeting of cells, a concept developed in Kimberlin & Walker’s (1983) theory of clinical target areas. In this theory clinical disease results from scrapie infection of specific brain areas. In support of this theory, stereotaxic injection of different areas of the brain resulted in different incubation periods (Kim et al., 1987b). The area of brain that gave the shortest incubation period differed with different scrapie strains. From the findings on genetic control of scrapie-induced obesity and altered glucose tolerance in the present study, it appears that there is also scrapie strain-specific targeting of cells involved in control of neuroendocrine functions.

In histological studies of scrapie-infected sheep brain, Beck et al. (1964) demonstrated severe bilateral symmetrical degeneration in the hypothalamic–neurohypophysial system with loss of cell bodies in the supraoptic nuclei and the paraventricular nuclei. Similar lesions were also present in the cerebellum, especially in the flocculo–nodular lobe, and in its afferent motor system, to which motor dysfunction may be attributed. On the basis of these findings, they suggested that scrapie in sheep could yield either motor or ‘metabolic and autonomic’ disturbance or, in some instances, a combination of both. In a subsequent study, using immunofluorescence histochemical techniques, Parry & Livett (1976) found an abnormal distribution of neurophysin and neurosecretory material in the proximal and distal portion of the hypothalamic–neurohypophysial system of scrapie-positive sheep. They also found increased levels of neurophysin in the adenohypophysis of these animals. This finding suggested to the authors that vasopressin might accompany the neurophysin and cause release of adrenocorticotrophic hormone. This provides a possible explanation for the adrenal cortical hypertrophy that had been noted previously (Beck et al., 1964).

In the present study the fact that the induction of changes in a neuroendocrine system occurred in a controlled and highly reproducible manner opens new directions for scrapie research. It seems unlikely that scrapie-induced changes in neuroendocrine systems will yield only aberrant glucose tolerance, obesity, adrenal hypertrophy (Carp et al., 1984; Kim et al., 1988) and ovarian pathology (Sturman, 1972). Rather, it seems probable that a variety of neuroendocrine systems will show changes and that the system affected and the types of changes will depend on the scrapie strain–host combination.

The authors wish to thank Jennifer Parese and Adele Monaco for their excellent assistance in preparation of the manuscript. The authors are also grateful to Richard Kascak and Richard Rubenstein for their review of the manuscript. This work was supported in part by NIH Grant No. NS21349 entitled ‘Characterization of scrapie-associated fibrils’.

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(Received 26 July 1988)