Interspecies transmission of Creutzfeldt–Jakob disease to Syrian hamsters with reference to clinical syndromes and strains of agent

(spongiform virus encephalopathy/slow viruses/scrapie/aging)

ELIAS E. MANUELIDIS, EDWARD J. GORACZ, AND LAURA MANUELIDIS

Communicated by Lewis Thomas, April 24, 1978

ABSTRACT  Experimental Creutzfeldt–Jakob disease was serially transmitted from guinea pigs to Syrian hamsters with 100% incidence, morbidity, and mortality. All animals developed a subacute spongiform virus encephalopathy with neuronal destruction and concomitant astrocytic changes. In the first passage three different clinical syndromes were recorded, each with widely variant incubation times; these results suggested there may be different strains of the Creutzfeldt–Jakob agent, some of which may be partially separated when the agent is passaged from one species to another. Accumulations of neurolipofuscin were suggestive of changes seen in senility and aging.

The "subacute spongiform virus encephalopathies" (1), characterized by degeneration of the central nervous system with neuronal loss, sponginess of the tissue (status spongiosus), and astrocytosis without accompanying inflammatory infiltrates, have been traced to unconventional replicating agents. In naturally occurring diseases these agents replicate in species ranging from human beings (kuru and Creutzfeldt–Jakob disease) to mink (transmissible mink encephalopathy) and sheep (scrapie). Because the exact nature of the agents comprising this group is unknown, the relationship between agents isolated from the naturally occurring diseases in different species and propagated in different experimental hosts forms the only basis for their comparison. Serial transmission of Creutzfeldt–Jakob disease of human beings to guinea pigs (2, 3), and more recently to mice (4), has been reported from this laboratory. The present paper reports the serial transmission of experimental Creutzfeldt–Jakob disease from guinea pigs to hamsters. Variations in the incubation periods, clinical syndromes, and histological lesions were observed. These results suggested the existence of different strains of the Creutzfeldt–Jakob agent. Experimental data reported in interspecies transmission of experimental scrapie are compared.

MATERIALS AND METHODS

Three young golden hamsters weighing 25–35 g were inoculated with 0.05 ml intracerebrally and 0.1 ml intraperitoneally of a 1:10 suspension of brain in physiological saline from two guinea pigs that had developed Creutzfeldt–Jakob disease during the fifth passage of the disease. Both guinea pigs used for inoculation revealed on histological examination the characteristic features of a spongiform virus encephalopathy.

For serial transmission, seven healthy hamsters (second passage) were injected with similarly prepared inoculum from a sick hamster of the first passage in the same quantities and routes of inoculation as that used in the first passage. When the hamster of the second passage developed the experimental disease (Table 1), a third group of six hamsters was similarly inoculated. From one sick hamster of the third passage the infection was passaged to six healthy hamsters, and the fourth passage is presently in progress. Routinely, half the brain of the sick hamster was frozen to be used for virological studies and the other half, including the spinal cord, was fixed en bloc in formalin for light microscopic investigations, including confirmation of the disease. Occasionally sick hamsters were perfused with formalin after anesthesia in order to obtain good fixation for light microscopy. All animals used for electron microscopy were perfused with Karnovsky's paraformaldehyde/glutaraldehyde fixative while alive, and representative sections were postfixed in osmium and embedded in Epon.

RESULTS

The three hamsters of the first passage developed three different clinical syndromes after widely varying incubation periods. The first hamster (paralyzed hamster), which was thinner than the other two, developed paralysis of the hind legs without any prodromal signs 34 days after inoculation. This animal was killed. The results of this transmission have been briefly reported (5). A second hamster (scratching hamster) developed, 3 months later, a scratching syndrome lasting for over 1 week; this hamster was excitable, ran in circles, and was killed 432 days after inoculation. The scratching was compulsive and stereotypic and consisted of repeated localized movement of the forelimbs over the lower cervical and upper thoracic region of the back. The skin became excoriated and covered with bloody scabs. Scratching at exactly the same region has been reported in mice infected with Creutzfeldt–Jakob material (4). After scratching, the hamster also licked and nibbled the paws of the forelimbs in a stereotyped fashion. The third hamster (prostrated hamster) was found lying on its back 3 months later, prostrate and unable to turn around; no prodromal signs prior to this event were detected. This hamster was killed 531 days after inoculation.

The second passage in hamsters resulted from inoculation of the brain of the first (paralyzed) hamster. One of seven animals died 1 day after inoculation and was discarded. The remaining six animals, in contrast to the three hamsters of the first passage that had displayed various syndromes, developed rather uniform clinical signs lasting for 1 week maximally and characterized by sluggish and uncoordinated movements, weakness, and ultimate prostration. In this second passage one animal, in addition, was observed dragging his hind limbs.

In the third passage six out of six hamsters became sluggish, unresponsive, lethargic, and ultimately prostrated without other prodromal signs. The maximal duration of sluggishness leading to prostration was up to 10 days. In the first three passages there was 100% susceptibility and morbidity (Table 1). The incubation period between the first and second passage was markedly reduced (by more than half) from an average 432.3 days in the
first passage to an average of 171.3 days during the second passage. The incubation period in the third passage became even shorter and averaged 132.8 days. All the animals of the second and third passages were moribund when killed.

The pathological findings in all three passages were entirely in the central nervous system; no abnormal changes were detected on microscopic examination of representative sections of the visceral tissues. Grossly, a minimal to moderate hydrocephalus ex vacuo was seen in all passages with the exception of the paralyzed hamster of the first passage. The histological changes in the central nervous system were as follows:

**Passage I. Paralyzed Hamster.** Conspicuous neuronal loss was observed in the anterior horn of the spinal cord in the lumbar region; in addition, a moderate increase of astrocytes and status spongiosus was also seen. The posterior horn of the lumbar region was similarly affected, but to a lesser degree. Qualitatively similar changes were detected in the cervical and thoracic spinal cord. In the cerebral cortex and in the basal nuclei a mild degree of neuronal loss, astrocystosis, and status spongiosus was observed. In addition, in the basal nuclei a mild increase in microglial cells could be seen. In dorsal parts of the pons a few vacuoles were seen in the neuropil and no changes were observed in the cerebellum.

**Scratching Hamster.** The status spongiosus in the cortex of the brain was considerably more striking than that seen in the paralyzed hamster and increased in intensity in sections proceeding from the frontal lobe (Fig. 1) to the posterior parietal and occipital regions. In the more posterior regions a striking vacuolization of the neuropil was evident (Fig. 2). A mild status spongiosus was present in the hippocampus, again more evident in posterior regions. A moderate status spongiosus was observed in the basal nuclei of the brain. Conspicuous vacuolization of the grey structures was observed in the corpora quadrigemina (Fig. 3), mesencephalon, pons, and medulla, in that order of decreasing intensity. The degree of neuronal cell loss and astrocystosis corresponded with the severity of vacuolization in the various regions of the central nervous system mentioned. Additionally, a mild increase of microglial cells was observed in the regions affected, especially in the basal nuclei of the brain. A few isolated vacuoles were seen in the granular cell layer of the cerebellum but this region was otherwise not affected. Status spongiosus of moderate degree was detected in the spinal cord more so in the cervical than in the thoracic and lumbar regions. However, the degree of neuronal loss described in the paralyzed hamster was not observed at any level of the spinal cord.

**Prostrate Hamster.** The distribution of the vacuoles was similar to that seen in the scratching hamster. Again, the status spongiosus was most conspicuous in the parieto-occipital region, corpora quadrigemina, mesencephalon, pons, and medulla.

![Fig. 1. Passage I, animal 2 (scratching hamster). Frontal cortex. Vacuolization (e.g., arrows), increased numbers of microglial cells, and neuronal loss can be seen. (Hematoxylin-eosin. ×450.)](image1)

![Fig. 2. Passage I, animal 2 (scratching hamster). Parietal cortex. Severe vacuolization and neuronal loss are present. (Hematoxylin-eosin. ×450.)](image2)

### Table 1. Three serial passages in hamsters

<table>
<thead>
<tr>
<th>Passage</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total inoculated</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total diseased</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>% incidence</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Incubation, days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal 1</td>
<td>334</td>
<td>161</td>
<td>115</td>
</tr>
<tr>
<td>2</td>
<td>432</td>
<td>164</td>
<td>125</td>
</tr>
<tr>
<td>3</td>
<td>531</td>
<td>166</td>
<td>125</td>
</tr>
<tr>
<td>4</td>
<td>173</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>175</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>189</td>
<td>146</td>
<td></td>
</tr>
<tr>
<td>Mean incubation, days ± SEM</td>
<td>432.3 ± 56.9</td>
<td>171.3 ± 4.2</td>
<td>132.8 ± 5.2</td>
</tr>
</tbody>
</table>
However, these regions were less affected than in the scratching hamster.

**Passage II.** Severe vacuolization of the neuropil, neuronal degeneration, and concomitant astrocystosis were observed predominantly in the posterior parietal and occipital regions of the cerebral cortex, in the corpora quadrigemina, mesencephalon, and medulla. By comparison, the lesions in the rest of the cerebral cortex, the basal nuclei, and spinal cord were mild to moderate. The cerebellum was only minimally affected.

**Passage III.** The distribution of the lesions was very similar to that seen during the second passage. Only mild quantitative differences were seen in individual animals during this passage.

Electron microscopic studies showed vacuolization predominantly in the dendrites and axons with less involvement of neuronal perikarya. Vacuoles of various sizes, partially encircled by membrane, were often seen in the nerve cell processes; they contained curled membrane fragments (Fig. 4) and occasionally a fine granular material. Many nerve cells revealed large numbers of lysosomes filled with material characteristic of lipofuscin. These were located both in the perikaryon (Fig. 5A) and in neuronal processes. Microglial cells were often identified in the vicinity of such neurons (Fig. 5A). A striking and unusual histological finding was the accumulation of dense neurofibrillary material in some identified neuronal cell bodies and processes (Fig. 5B). These were 90–100 Å in diameter. We have not observed these filaments in a twisted configuration, as seen in human Alzheimer’s disease. Reactive astrocytes packed with glial filaments were found throughout the affected regions (Fig. 6).

In control hamsters inoculated with normal guinea pig brain by similar routes and with identical dosages as the hamsters inoculated with the infectious material no disease developed and no spongiform changes were seen in the brain. Examination of age-matched controls and older hamsters (over twice the age of the infected animals) never revealed the neurofibrillary accumulations seen in the Creutzfeldt-Jakob material. Lipofuscin accumulations (lysosomes) were, however, observed in aged control hamsters also, although to a lesser degree.

**DISCUSSION**

The present experiments demonstrate the successful transmission of experimental Creutzfeldt-Jakob disease from guinea pigs to an additional convenient host, the hamster. Like guinea pigs (3), the hamsters showed 100% susceptibility, morbidity, and mortality in the dilutions and routes of inoculations indicated.

The incubation time of experimental Creutzfeldt-Jakob disease in hamsters during the second passage decreased by half (Table 1) and continued to decrease during the third passage. Whether the incubation period will level off in subsequent passages, as has been reported in guinea pigs (3), remains to be seen. Recently, Kimberlin and Walker (6) reported in golden hamsters a short incubation (60 days) of scrapie after the fourth passage by repeated passages of the Chandler mouse agent of scrapie into hamsters.

A mild to moderate dilatation of the ventricular system of the brain was observed in many hamsters, especially during the second and third passages. This *ex vacuo* or compensatory hydrocephalus was interpreted, as in the guinea pigs with Creutzfeldt-Jakob disease (3), to be a result of, or secondary to, severe neuronal devastation. The lesions observed in the central nervous system of infected hamsters, namely, status spongiosus, neuronal degeneration, and astrocytic changes, were similar...
to those seen in guinea pigs with experimental Creutzfeldt-Jakob disease (3). However, additional striking alterations were seen in electron micrographs. In some neuronal perikarya and dendrites many lysosomes with lipofuscin as well as dense neurofibrillary accumulations were seen; both these changes are often associated with, and cardinal features of, senile brains or aging. Astrocytic gliosis was also a more prominent feature in hamsters.

The distribution of the lesions within the central nervous system in the serially transmitted Creutzfeldt-Jakob disease in hamsters is different from that observed when this disease was transmitted to guinea pigs. In guinea pigs the lesions were mainly in the cerebral cortex without particular predilection of any cerebral lobe and in the basal nuclei (3). The lesions in the mesencephalon, pons, and medulla were by comparison mild, and for all practical purposes no changes were seen in the cerebellum and spinal cord. When Creutzfeldt-Jakob disease was serially transferred in the present experiments to hamsters, the predilection and the brunt of the lesions were in the parieto-occipital region of the brain, mesencephalon, pons, and medulla. Furthermore, there was involvement of the spinal cord and minimal changes in the cerebellum. Zlotnik (7) and Zlotnik and Rennie (8) were the first to transmit scrapie to golden hamsters. The nature of the lesions produced was similar to that seen in hamsters infected with the Creutzfeldt-Jakob agent; however, the predilection for and distribution of the lesions in particular regions of the central nervous system were not recorded (7) although it was reported that “neuronal degeneration, single and multiple neuron vacuolation and status spongiosus were very widespread” (8). In a study of scrapie and transmissible mink encephalopathy in hamsters, Marsh and Kimberlin reported that scrapie-inoculated hamsters that died early (17–18 weeks) after inoculation revealed intense involvement of brainstem and cerebellum and moderate lesions in the cerebrum; the opposite was true in hamsters inoculated with transmissible mink encephalopathy that died early (22 weeks) after inoculation (9). The pathological changes in scrapie

FIG. 5. (A) Passage II, animal 3. Parietal cortex. Neuronal cell body contains many lysosomes with lipofuscin (L). A vacuole (V) is additionally noted in the cytoplasm. Adjacent to this neuron is a microglial cell (M). (X13,487.) (B) Passage II, animal 3. Parietal cortex. Accumulations of fibrils (f) in two regions of dendrite receiving synaptic input(s) are seen. Note also abnormal accumulation of dense and membranous material in a cross section of myelinated axon (a). (X9900.)

FIG. 6. Passage II, animal 3. Parietal cortex. Reactive astrocyte packed with fibrils (f) is noted. (X10,210.)
and transmissible mink encephalopathy became gradually indistinguishable in animals dying after extended periods of time (9).

The variations in the clinical and pathological findings reported in the present serial transmissions can best be understood and interpreted in relation to pertinent and important data on interspecies transmission of scrapie. Pattison and Millson (10) were the first to suggest the existence of different strains of the scrapie agent when they reported two distinct clinical syndromes, the "nervous or drowsy" and the "scratching" syndromes in goats inoculated with sheep scrapie. Inoculations of the "scratching" and "drowsy" materials into goats, sheep, and mice in subsequent experiments (11) shows that each isolate from these different syndromes (strains) in serial passage continued to reproduce its own clinical syndrome; in mice, however, the "drowsy" inoculum produced only a shorter incubation period than the "scratching" inoculum, but not a different syndrome (11). Subsequent investigations by Dickinson and associates, using an array of inbred and F1 mice, established that the "scratching" infectious material contained two strains of scrapie, 22C and 80V, and the more extensively analyzed "drowsy" material contained at least three strains, 79V, 139A, and 79A (12–15).

At least partial separation of the strains of agent apparently also occurs in scrapie when material is passed from one host to another. Pattison and Jones reported a permanent change in clinical signs and microscopic lesions when the mouse-passaged Chandler agent was passed five times through rats and four times through mice (16). There is information that the mouse Chandler agent was originally a mixture of scrapie strains (A. G. Dickinson, unpublished data). In typing the various strains of the scrapie agent in mice with different stic genotypes two basic techniques were used, namely, absolute and relative differences in the incubation periods (14, 17, 18) and quantitative and qualitative profiles of lesions in the brains of the inoculated mice (15, 18, 19).

The most unusual and striking observations in the present investigations were recorded during the first passage of Creutzfeldt–Jakob disease from the guinea pigs to the hamsters. Three different clinical syndromes were seen in the first passage with paralyzed, scratching, and prostrated hamsters. The incubation periods in these animals were 334, 432, and 541 days, respectively. The variety of clinical syndromes and the marked differences in the incubation periods, namely, 3 months apart, obtained during the first passage into hamsters strongly suggest that the inoculum from the fifth guinea pig passage was heterogeneous and contained a mixture of strains of the Creutzfeldt–Jakob agent.

In the second and third passages the incubation periods of the individual animals, unlike the first passage, were close (Table 1). Furthermore, the clinical signs were more uniform and the distribution of the lesions in the brain during the second and third passages to hamsters showed only minor, insignificant quantitative differences in individual animals. By analogy to scrapie, these events again indicate that there may be a possible partial separation or selection of strains of the Creutzfeldt–Jakob agent when the infection is transferred from guinea pigs into hamsters.

Cases of human beings with Creutzfeldt–Jakob disease have been published under at least twenty different designations and subgrouped into five (20) or six (21) nosological entities according to the regions of the central nervous system affected and the resulting clinical symptomatology. The duration of the human illness varies from patient to patient (22), some lasting one to several months (type I), others less than 9 months (type II), and still others lasting as long as 1–2 years (type III). The present investigation in hamsters strongly suggests that some of these Creutzfeldt–Jakob subgroups, displaying different syndromes and different durations of human disease, may be related to the action of different strains of the Creutzfeldt–Jakob agent. Successful experimental transmission of representative cases of Creutzfeldt–Jakob disease and typing of various strains of the agent, by using techniques so successfully applied in scrapie research (18), may eventually substantiate this notion.

Addendum. After completion of our present manuscript, Richard H. Kimberlin kindly supplied us with a preprint entitled "Evidence that the transmission of one source of scrapie agent to hamsters involves separation of agent strains from a mixture" [Kimberlin, R. H. & Walker, C. A. (1978) J. Gen. Virol., in press]. The results of this study indicate the presence of at least two strains of the scrapie agent at the third passage level in the brain of outbred golden hamsters. One of these strains (431K) is highly pathogenic for mice and the other (263K) has an extremely low pathogenicity for mice. However, only one of these strains (263K) is present in hamster brain after the sixth serial passage. It is suggested that the "adaptation" of scrapie to hamsters may involve the selection of a single strain, from a mixture, that is highly pathogenic for hamsters. The results with scrapie support our observations as well as the views expressed in the present study on Creutzfeldt–Jakob disease in hamsters.

We express our grateful thanks for the dedicated help of Susan Valley, Joseph N. Musco, Elizabeth A. Mullaly, and Ronald Nicholes. This work was sponsored by U.S. Public Health Service Grant NS-12674.