Serial propagation of Creutzfeldt–Jakob disease in guinea pigs
(human/Slow viruses/spongiform virus encephalopathy/cerebral atrophy/electron microscopy)

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Communicated by Lewis Thomas, September 25, 1975

ABSTRACT The transmission and serial propagation of Creutzfeldt–Jakob disease from man to guinea pigs are reported. The latency, symptomatology, and morphology of the infection during the first four passages are presented. The incubation period between the first and subsequent passages was halved. One hundred percent take, morbidity, and mortality were achieved in all inoculated animals. All guinea pigs developed a subacute spongiform virus encephalopathy with marked neuronal destruction in the cerebral cortex and subcortical grey structures. The neuronal loss resulted in cerebral atrophy and hydrocephalus ex vacuo.

The group of “subacute spongiform virus encephalopathies” (1) includes four naturally occurring diseases, kuru and Creutzfeldt–Jakob disease of man, scrapie of the sheep, and transmissible mink encephalopathy. Scrapie was the first and the most extensively studied of these encephalopathies and the transmission of scrapie from sheep to mice (2) provided a convenient experimental model for the investigation of this disease. Kuru, an exotic subacute neurological disorder occurring in New Guinea, was transmitted from man to three chimpanzees (3). Subsequently, Creutzfeldt–Jakob disease, a progressive neurological disorder classified as a “degenerative” disease was also successfully transmitted to the chimpanzee (4). Although both kuru and Creutzfeldt–Jakob disease have been transmitted additionally to Old World and New World monkeys, and Creutzfeldt–Jakob disease to domestic cats (5), transmission of these two human diseases to small laboratory animals such as mice, rats, hamsters, and guinea pigs has failed (5) even though immunosuppressive drugs, X-irradiation, splenectomy, and thymectomy were additionally used (6). In a recent abstract the passage of Creutzfeldt–Jakob disease from a human biopsy to a group of mice has been reported (7).

The present study deals with the transmission of Creutzfeldt–Jakob disease from a human biopsy to guinea pigs during the first four serial passages. Three guinea pigs (Hartley strain) 2–3 months old were inoculated with 0.1 ml intracerebrally and 0.2 ml intraperitoneally of 1:8 suspension of the fresh human brain biopsy in normal saline. For the intracerebral inoculation the same technique was employed as that used for many years in this laboratory for the heterologous transplantation of human tumors (8). A sick guinea pig from the first passage was used for the inoculation of healthy guinea pigs (second passage) injected with 0.1 ml intracerebrally and 0.2 ml intraperitoneally of a 10−1 brain suspension in normal saline. When these animals developed the disease three groups of guinea pigs (third passage) were similarly inoculated from three different sick guinea pigs (Table 1). Similarly, the disease was subsequently transferred from one of these guinea pigs to the fourth passage. At the present time, the fifth passage of the serial transmission of Creutzfeldt–Jakob disease to guinea pigs is in progress.

With the exception of the first passage, all animals used for electron microscopic studies were perfused with Karnovsky’s paraformaldehyde–glutaraldehyde fixative while alive and representative sections were then exposed to osmium and embedded in Epon. Guinea pigs to be studied by light microscopy were perfused with 10% neutral formalin in order to avoid artefactual distortion of tissue. Half of the brain of the animals used to transfer the disease to another group of healthy guinea pigs was always taken for histological confirmation, and fixed en bloc in formalin.

RESULTS

Of the three guinea pigs inoculated with human brain biopsy suspension, one died shortly after inoculation and was discarded. Over 1 year later, the other two developed a neurological disorder characterized by increasing agitation, running in circles, grinding of the teeth, weakness of the extremities, and ultimately prostration. The apparent onset of the disease in the first passage as well as in the subsequent three serial passages that also resulted in similar progressive neurological symptoms is given in Table 1 and graphically illustrated in Fig. 2. It can be seen that the incubation period of Creutzfeldt–Jakob disease was markedly reduced between the first and second passage in guinea pigs; an average of 467 days for development of debilitating neurologic disease was obtained for the first passage and this was more than halved to an average of 216.5 days during the second passage. In the three groups of the third passage this incubation period for all practical purposes leveled off (Fig. 2). By comparison with the very long incubation period without any detectable clinical signs, the duration of the disease was short and lasted maximally 10 days. The susceptibility of the animals as well as the mortality to the infection was 100%, when they were inoculated via the combined intracerebral and intraperitoneal routes in the amounts and in the dilu-
tions indicated above. If the inoculated guinea pigs were not sacrificed they died within days as a result of Creutzfeldt–Jakob disease.

The pathological findings were restricted to the central nervous system and examination of the visceral organs with the light microscope failed to reveal any pathological changes. Grossly at autopsy the most conspicuous and consis-
tent finding was a moderate to a marked hydrocephalus (Fig. 3). Whenever the enlargement of the ventricular system was seen in the inoculated guinea pigs, positive microscopic and ultrastructural findings were expected and were subsequently found. The microscopic alterations consisted of status spongiosus in the cerebral cortex and in the subcortical grey structures. Neuronal loss was evident in the affected regions and the remaining nerve cells showed vacuole formation in their cytoplasm (Fig. 4). Cajal’s stain for astrocytes showed an increased number of hypertrophic astrocytes in both cortex and basal ganglia (Fig. 5). Occasionally, in the subcortical structures there was a mild proliferation of elongated microglia cells. Infiltrates composed of lymphocytes and plasma cells were at no time seen and no demyelinating lesions were observed.

Ultrastructural studies showed vacuolization of neurons preferentially affecting the dendrites and the axons (Figs. 6 and 7). The vacuoles were membrane-bound and were either empty or contained curled fragments of membranes and/or fine granular material. In some instances discontinuities and ruptures of the membranes were noted. In addition, focal swelling and clearing of the cytoplasm of the nerve

![Image](https://example.com/image.png)

**FIG. 2.** Marked reduction in the time of clinical onset of the disease from the first to subsequent passages. Open circles represent the mean and standard error of the mean is indicated. Black circles represent individual animals in first passage.

### Table 1. Data for four serial passages in guinea pigs

<table>
<thead>
<tr>
<th>Passage</th>
<th>I</th>
<th>II</th>
<th>III-A</th>
<th>III-B</th>
<th>III-C</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total inoculated</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>12</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Total diseased</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>12</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>% take</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Incubation/animal, days</td>
<td>1.422</td>
<td>1.187</td>
<td>1.244</td>
<td>1.193</td>
<td>1.207</td>
<td>1.192</td>
</tr>
<tr>
<td>Mean incubation, days ± SEM</td>
<td>467 ± 45</td>
<td>216.5 ± 6.7</td>
<td>247 ± 2.4</td>
<td>220.9 ± 3.3</td>
<td>236.2 ± 4.4</td>
<td>202.3 ± 3.7</td>
</tr>
</tbody>
</table>

Passage III-A, -B, and -C represent inoculations from three different sick animals of passage II.
cells were seen associated with herniation of the affected cytoplasm and on occasion rupture of the plasma membrane. Swelling of the astrocytes was occasionally observed; however, vacuolization as seen in the neurons and their processes could not be detected. Oligodendrocytes did not reveal any changes. Occasionally phagocytizing microglia cells were noted. Again no perivascular infiltrates or primary demyelination could be seen. No known viruses or virus-like particles were seen in the numerous sections examined.

DISCUSSION
Since the fundamental discovery by Gibbs, Gajdusek, and coworkers that the Creutzfeldt–Jakob disease is a transmissible encephalopathy (4) several unsuccessful attempts were undertaken to passage this disorder to rodents. The pres-
ent results demonstrate the successful transmission of Creutzfeldt-Jakob disease from a human biopsy to the guinea pig during four serial passages over the past 3 years. Guinea pigs revealed 100% morbidity and mortality in all serial passages with the indicated dilutions and routes of inoculation. Thus this species constitutes a very convenient experimental model for the study of the biological properties of the agent and for the pathogenesis of Creutzfeldt-Jakob disease. It is difficult to explain why this disease was passaged to the guinea pigs, whereas attempts up to this time in other laboratories have failed. It may very well be that, as with conventional viruses, the age and the specific strain of the host are of some importance in the transmission of subacute spongiform virus encephalopathies. It is unlikely that the combined routes of inoculation (intracerebral and intraperitoneal) have anything to do with the present successful transmission of the disease to guinea pigs, since in subsequent experiments in this laboratory intracerebral inoculations alone caused the disease without any change in the incubation period. A second possibility is that the use of fresh rather than frozen biopsy material had something to do with the transmission of the disease to rodents, as the recent successful transmission of Creutzfeldt-Jakob disease from a human biopsy to mice also indicates (7). Yet, transmission of this disease to primates after storage of the inoculum for several months at −70°C is possible (9).

The marked reduction of the incubation period between the first and the second passage in the experimental disease in the guinea pig was not surprising (see Fig. 2). A similar event has previously been reported in human spongiform virus encephalopathies transmitted to primates (9).

Anatomically, the most consistent and conspicuous gross finding was the moderate to marked dilatation of the ventricular system. In the inoculated animals this ex vacuo or compensatory hydrocephalus was pathognomonic, since positive histological findings were always subsequently found. This dilatation is most likely the result of the neuronal devastation observed in the cortex and the subcortical grey structures of stricken animals. Such gross findings apparently were less striking in others’ experimental transmission of human spongiform virus encephalopathies. Thus, in experimental kuru in chimpanzees, in a total of 29 animals, five showed slight atrophy of the vermis of the cerebellum and three others revealed slight dilatation of the lateral ventricles (10). In experimental Creutzfeldt-Jakob disease in the same host only slight cortical atrophy over the vertex but not ventricular dilatation was reported (11). Qualitatively the histological and ultrastructural findings in the brain of the guinea pigs were similar to those seen in subacute spongiform virus encephalopathies which have been reviewed recently (12). The variations on the theme and the distributions of the lesions within the neuraxis of the guinea pig will be reported in detail elsewhere.

The authors express their grateful thanks for the dedicated help of Miss Phyllis Johnson. This work was sponsored by USPHS Grants NS-12574-01 and CA-15044-02.