# Infection as the Etiology of Spongiform Encephalopathy (Creutzfeldt-Jakob Disease)

Abstract. *Fatal spongiform encephalopathy occurred in four chimpanzees 12 to 14 months after inoculation with suspensions of brain from four patients, respectively. Chimpanzee to chimpanzee transmission was effected without reduction in incubation period. Retransmission of the disease to a second chimpanzee occurred when an inoculum that had been stored at −70°C for over 2 years was used.*

Transmission of spongiform encephalopathy, or Creutzfeldt-Jakob disease, to a chimpanzee inoculated with a suspension of brain biopsy tissue, obtained from a patient 5 months before his death (*1*), has prompted attempts at retransmission, serial passage of the disease from chimpanzee to chimpanzee, and further primary transmissions with brain obtained from other patients with the same disease. Chimpanzees have been inoculated intracerebrally and intravenously with brain suspensions from the original patient and from eight additional patients. Other chimpanzees have received similar inoculations with brain suspension from the first two animals to develop the disease.

Both retransmission and serial passage from chimpanzee to chimpanzee have been successful. In addition, we have transmitted the disease to three other chimpanzees, each inoculated with a suspension of brain from one of three new patients with spongiform encephalopathy. The inoculations and serial passages from the four patients whose brain tissues have caused disease in the chimpanzees are summarized in Fig. 1.

To date, six animals have developed the disease, all of them 12 to 14 months after inoculation of 0.2 ml intracerebrally and 0.3 ml intravenously of 5 or 10 percent suspensions of brain. These include (i) A54, the animal of the first reported transmission from a British patient; (ii) A82, the animal inoculated with brain suspension from chimpanzee A54; (iii) A79, the animal used for retransmission, inoculated with the same brain tissue that had first caused the disease and that had been stored at −70°C for over 2 years; (iv and v) two animals, A77 and A78, that received brain tissue of two American patients, respectively, taken at autopsy performed soon after death; and (vi) A81, an animal inoculated with a suspension of brain tissue from a second British patient.

The five additional chimpanzees, still well after inoculation of brain suspension from a different one of five other patients with spongiform encephalopathy, were inoculated subsequent to the six chimpanzees that have now developed the disease; and only two of these five have yet been observed for 12 months, which is the minimum incubation period observed in the six animals that have developed the disease.

The four patients, each in the sixth decade of life, whose brain tissues have thus far caused similar fatal spongiform encephalopathy in the chimpanzees, have each died from an unremitting and rapidly progressive brain disease with associated severe dementia, disturbances of vision, myoclonic jerks, and ataxia. All four were diagnosed clinically as suffering from Creutzfeldt-Jakob disease; this was confirmed in the first patient by a brain biopsy that showed marked status spongiosis of cortical gray matter. The duration of disease in these four patients was 8, 2½, 10, and 5 months, respectively. At autopsy, neuropathological study revealed the typical pathology of Creutzfeldt-Jakob disease of extensive spongiform encephalopathy of gray matter, neuronal loss, and associated astrogliosis (*2*).

All six affected chimpanzees, including the second passage animal, have developed a remarkably similar disease (after incubation periods of 12 to 14 months) in which the major clinical features have been tremor with associated ataxia, myoclonic jerking, fasciculation, somnolence, visual disturbances, and dementia. The illness has been rapidly progressive, leading to total incapacitation in about 2 months. Interestingly, the disease is clearly distinguishable from experimental kuru in the chimpanzee (*3, 4*) in its clinical aspects, particularly because of the severe dementia, myoclonus, fasciculations, and somnolence, none of which are prominent features of experimental kuru. Clinical summaries of these six chimpanzees are given below.

Chimpanzee A54, a juvenile male animal, inoculated on 20 November 1966 with 5 percent suspension of brain biopsy material from patient 1, a British patient, has been described in detail previously (*1*). The animal was killed 2 months after onset when lethargy, tremors, myoclonus, ataxia, right hemiparesis, dementia, and difficulty with vision had progressed to a level of severe incapacitation. Pathological examination, reported elsewhere (*5*), showed neuronal loss, status spongiosis of gray matter, and hypertrophy and proliferation of astrocytes with some microglial reaction similar to that seen in the patient.

Chimpanzee A77, a juvenile female animal, was inoculated on 2 February 1968 with 10 percent suspension of brain material in phosphate-buffered saline (*p*H 7.4), 0.2 ml intracerebrally and 0.3 ml intravenously. The animal remained well until early February 1969, 12 months after inoculation, when slight tremors of the trunk and extremities were noted, which resulted in instability of gait. This progressed slowly until mid-March when the ataxia became severe and the tremors, about two per second in frequency, had the regularity and severity of myoclonus. The animal could no longer stand or walk without use of the anterior extremities, and used these extremities to prevent falling over when seated. She appeared to have poor vision and to be confused in seeing familiar objects. The animal was killed on 27 March 1969, 13 months after the onset of symptoms, by exsanguination. Autopsy was immediately performed, with brain tissue taken for electron microscopy, frozen section pathology, and virological and immunological studies, the remainder of the brain being fixed in 10 percent formol-saline for histopathology. Preliminary sections, courtesy of Dr. Peter Lampert, have shown neuronal loss, spongiform changes of the cerebral cortical gray matter and the basal ganglia, and an associated astrocytosis, a pathology similar to that in the patient.

Chimpanzee A78, a juvenile female, inoculated on 2 February 1968 with 10 percent brain in buffered saline, remained well until early April 1969. At that time she developed generalized tremors of intention type when feeding and difficulties in walking, with an abnormal high-stepping gait, particularly involving the right leg. Within 1 week of the onset of signs, she developed fasciculations of upper and lower extremities and myoclonus involving the extremities and the whole body, with massive jerks occurring infrequently at

intervals of 4 to 8 minutes. She seemed lethargic, disoriented and confused, acting as though she did not see things well. She developed brief seizure-like episodes of unconsciousness, ending in a jerk, fasciculations in all extremities, and hyperactive reflexes, predominantly on the right. Her disease progressed rapidly and she walked only with extreme difficulty, preferring to remain lying inert and confused, and developed a severe right-sided spastic hemiplegia. She was killed on 13 June 1969.

Chimpanzee A79, a juvenile female, inoculated intracerebrally and intravenously on 22 March 1968 with the same suspension of human brain (patient 1, R.R.) used to inoculate chimpanzee A54 in November 1966, remained asymptomatic until 1 year later, when she seemed to have a slightly abnormal gait. By early April, tremors of the head, trunk, and extremities were apparent, and there was an abnormal, clumsy gait and mild right-sided hemiplegia. She showed signs of confusion and disorientation and appeared lethargic and withdrawn. Her symptoms progressed and 2 months after the onset of signs she became completely inert; apparently blind, showing no response to a lighted match or apple held before her; lethargic, with a spastic quadriplegia and fasciculations in all extremities; and hyperactive, deep, and superficial reflexes. She produced an abnormal, high-pitched scream when disturbed. She was killed in this advanced stage of her illness, at 2½ months after onset of signs.

Chimpanzee A82, a juvenile female, inoculated on 22 March 1968, intracerebrally and intravenously, with a 10 percent suspension of brain from chimpanzee A54, was killed in the terminal stages of experimental disease. Thirteen months after inoculation A82 showed signs of inattention, listlessness, irritability, withdrawal, and lethargy; a head and body tremor appeared, and short episodes of myoclonus involving the whole body were noted soon thereafter. There was difficulty with gait, associated with the tremors, and mild right-sided spastic hemiplegia. Confusion and lethargy progressed to somnolence. She appeared to be unable to recognize objects shown to her. She deteriorated rapidly, developed a spastic quadriplegia, and was totally incapacitated in a flexed posture 1½ months after the onset of the disease. Cerebrospinal fluid showed no pleocytosis nor elevation of protein. She was killed 6 weeks after the onset by exsanguination and autopsy was performed as described for chimpanzee A54. Preliminary neuropathological examination by Dr. Peter Lampert has shown the same sort of neuronal loss, spongiform change of cerebral cortical gray matter and the basal ganglia, and gliosis as was seen in chimpanzees A54 and A79 and in the patient from whom the disease had been transmitted.

Chimpanzee A81, a juvenile male animal, was inoculated 22 March 1968 with 10 percent suspension of brain autopsy material from patient 4, a British patient, in phosphate-buffered saline (*p*H 7.4), 0.2 ml intracerebrally and 0.5 ml intravenously. The animal remained well until 29 May 1969, 14 months after inoculation, when lethargy and tremors of head, arms, and lower extremities were noted. Within 1 week of the onset of signs, he developed myoclonic jerks of total body and head, and individually of legs and arms. These were quick and occurred in series. The animal was also clumsy and tremulous. Two weeks after onset chimpanzee A81 became confused and had difficulty in associating familiar objects and surroundings; he startled very easily and any movements in his direction evoked high-pitched cries. A right-sided weakness developed and there was increased tone and hyperactive reflexes. By the 3rd week the animal had developed a profound hemiparesis and efforts to walk caused stumbling and

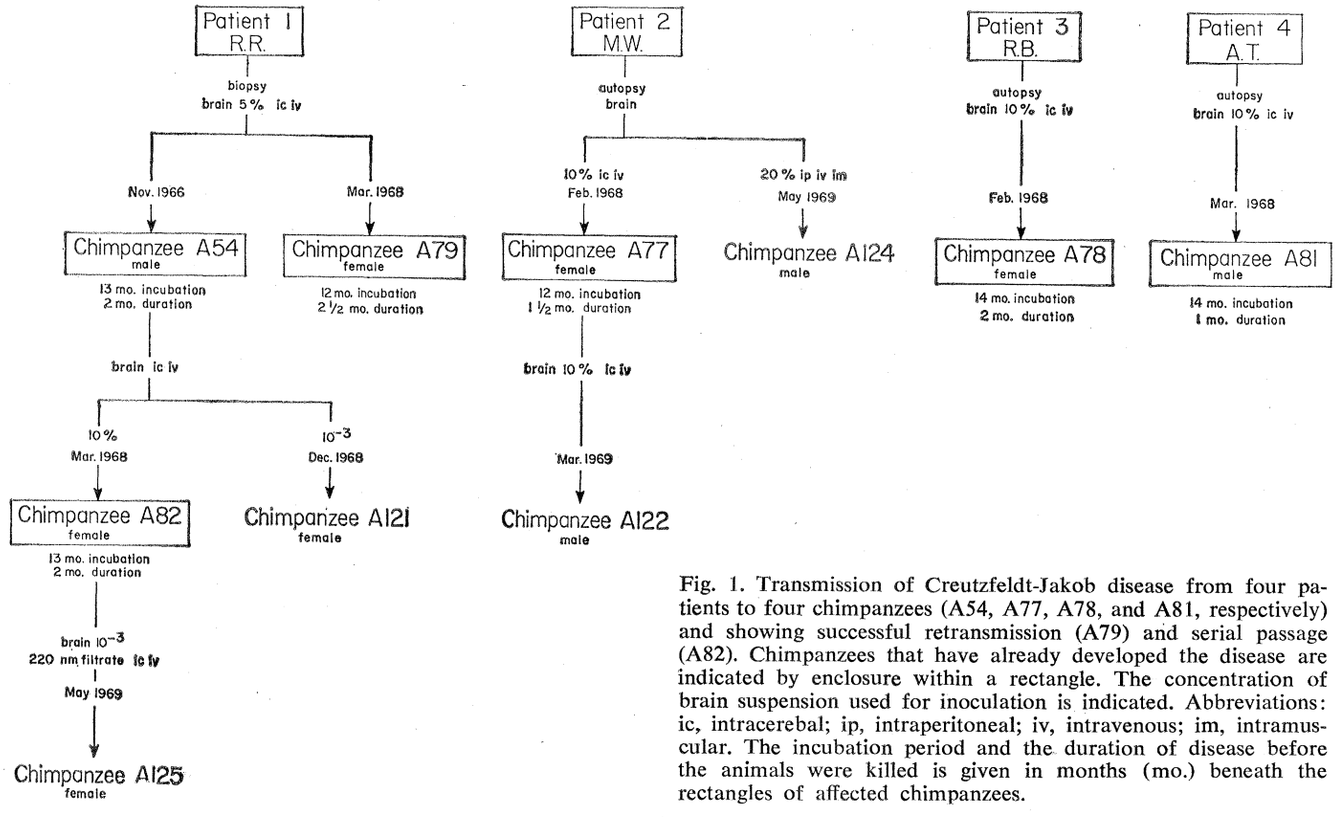


Fig. 1. Transmission of Creutzfeldt-Jakob disease from four patients to four chimpanzees (A54, A77, A78, and A81, respectively) and showing successful retransmission (A79) and serial passage (A82). Chimpanzees that have already developed the disease are indicated by enclosure within a rectangle. The concentration of brain suspension used for inoculation is indicated. Abbreviations: ic, intracerebal; ip, intraperitoneal; iv, intravenous; im, intramuscular. The incubation period and the duration of disease before the animals were killed is given in months (mo.) beneath the rectangles of affected chimpanzees.

falling. The right side was not used voluntarily. The right side of the face drooped, eyes were tonically deviated to the left, and a right hemianopsia field defect to light and threat was noted. Marked tremors were noted bilaterally with intention. No fasciculations were seen. Deep tendon jerks were 4+ with tap clonus of patella. No Babinski was elicited. The jerks and general state continued downhill, with the animal in a flexed position on the bottom of the cage. During the 5th week of clinical disease myoclonic jerks intensified and a resting continuous tremor of the lips, lower jaw, and head was noted. The animal was killed on 7 July 1969.

Neuropathologic and electron microscopic examination of the brains of the first three animals that we killed after they developed disease revealed a vacuolation of the dendritic and axonal processes in neurons and in the astroglial cells, as well as the presence of large rounded neurons containing a pale cytoplasmic inclusion body (*5, 6*).

Although the same suspensions, from human and affected chimpanzee brains, that have caused disease in the chimpanzees have been inoculated into several other species of primates and other small laboratory animals, including suckling mice and hamsters, no disease has developed in these animals after over 1 year of observation. Since kuru has also been successfully transmitted to the spider monkey (*7*), this species has been used for these inoculations. A chimpanzee, A124, has received a suspension of infectious human brain from patient 2 (M.W.) only by peripheral (intravenous, intraperitoneal, or intramuscular), not intracerebral, routes of inoculation; another animal, A125, has been inoculated in third passage with a filtrate of brain suspension from chimpanzee A82 at 1:1000 dilution, which had been passed through a cellulose acetate (Millipore) membrane, (average pore diameter, 220 nm).

The spongiform encephalopathies include principally a group of subacute presenile dementias, usually called Creutzfeldt-Jakob disease. The basic pathological change is a vacuolization in the dendritic and axonal processes of neurons, and to a lesser extent, in glial elements (*7*). Kuru in New Guineans is associated with the same spongiform degeneration of neurons. Alpers disease, or progressive diffuse cerebral degeneration of infants, may also possibly belong to the group, in view of its pathological similarities. In animals, scrapie and mink encephalopathy show the same process, but in natural scrapie it progresses only to neuronal vacuolation, and not to a full status spongiosis of cerebral gray matter. In scrapie, experimentally transmitted to sheep, goats, mice, rats, hamsters, or gerbils, however, as in experimentally transmitted mink encephalopathy, the spongiform degeneration becomes the dominant picture. The same is true of the spongiform degeneration in experimental kuru and experimental Creutzfeldt-Jakob disease, which exceeds that seen in the natural diseases.

These successful transmissions of Creutzfeldt-Jakob disease with spongiform encephalopathy of gray matter suggest that the disease of these patients should be included with the virus infections which may be designated the subacute spongiform viral encephalopathies: Creutzfeldt-Jakob disease, kuru, scrapie, and mink encephalopathy.

*Note added in proof*: Since this report was submitted, two of the five chimpanzees referred to above (A106 and A114), inoculated with brain suspension from a Canadian patient and a third British patient, respectively, have developed clinical signs similar to those observed in chimpanzees already killed in advanced stages of induced Creutzfeldt-Jakob disease. Transmission has thus been successfully accomplished with brain suspension from each of six of the eight patients referred to in this report. Further, a variety of tissues obtained at surgical biopsy and autopsy from an additional ten patients, from the United States, Canada, and Great Britain, with Creutzfeldt-Jakob disease, are under study.

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**References and Notes**

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## Erythropoiesis in the Dog: The Periodic Nature of the Steady State

Abstract. *In at least six of 11 normal dogs serial measurement of reticulocyte counts showed oscillation with a period of approximately 14 days. The phase of oscillation could be altered by bleeding followed by retransfusion. The observations suggest that canine erythropoiesis is an example of a physiological rhythm which has its origin in homeostatic control.*

Many body parameters are known to be actively controlled in such a way as to oppose disturbances and result in a more or less steady state. A commonly assumed and expressed corollary to this concept of active regulation is that a perfectly steady state results when no external disturbances are acting. However, clear exceptions to this corollary exist. The steady states of the pituitary-adrenal axis and, in the female, the pituitary-gonadal axis are sustained oscillations. On a cellular scale the activities of a number of biochemical intermediates have been shown to oscillate constantly (*1*). Recently, evidence was produced which showed that granulopoiesis is another physiological system whose steady state is one of sustained oscillation (*2*). It was suggested that it is controlled by a negative feedback circuit containing a time-delay and that periodicity arises because this type of circuit is inherently prone to oscillate.

Erythropoiesis is controlled by a hormone erythropoietin whose plasma level is a function of circulating red cell hemoglobin and whose principal action is to induce erythropoietin-sensitive stem cells to differentiate into recognizable erythroid precursors (*3*). These precursors must proliferate and mature before they can enter the blood