Creutzfeldt-Jakob Disease (Spongiform Encephalopathy): Transmission to the Chimpanzee

Abstract. *Biopsy material taken from the brain of a patient with Creutzfeldt-Jakob disease with status spongiosus induced a similar fatal encephalopathy in a chimpanzee 13 months after inoculation.*

Transmission of kuru to the chimpanzee (*1*) has stimulated a search for other subacute degenerative conditions of the central nervous system that, when inoculated into primates, may induce neurological disease. Kuru has been transmitted from each of eight human brains to chimpanzees; the agent is serially transmissible, but the experimental range of hosts is still restricted to the chimpanzee (*2*). Attempts to transmit other neurological diseases were unsuccessful (*2*) until recently.

Our purpose is to describe transmission to the chimpanzee of a second such disease, resembling kuru pathologically (*3*), from a patient with severe status spongiosus of the cerebral cortical gray matter. Such status spongiosus is found in several brain diseases, including kuru; it is exceptionally severe in chimpanzees with experimentally induced kuru. It is found most often in patients in whom Creutzfeldt-Jakob disease is diagnosed.

Creutzfeldt-Jakob disease is generally conceded to be an ill-defined term for a group of subacute presenile encephalopathies characterized clinically by dementia, involuntary movements (myoclonic jerks), and other less constant findings that often include ataxia. Because of the variability of pathological findings in these patients, several authorities have tried to group together smaller numbers of cases sharing common neuropathological features. In a series described by Nevin *et al.* (*4*) most cases showed status spongiosus of the cerebral gray matter and indirect evidence of vascular dysfunction. These authors believed that their cases represented a disease entity that should be excluded from Creutzfeldt-Jakob disease; they suggested the term subacute spongiform encephalopathy. Brownell and Oppenheimer (*5*) reviewed a group of patients having selective degeneration of cerebellar granule cells, variable status spongiosus, and other findings, but showing no evidence of vascular dysfunction; they considered them to have a form of Creutzfeldt-Jakob disease.

Our patient was a 59-year-old white man with an unremitting and progressive brain disease of 8-month duration; he had severe dementia, myoclonic jerks, and other signs that are discussed below (*6*). A brain biopsy performed 3 months after the onset of illness showed marked status spongiosus of cortical gray matter. This finding suggested that the descriptive diagnosis of spongiform encephalopathy might be appropriate. However, at death, 5 months after biopsy, the brain showed complete replacement of the spongy state by severe "collapsed-appearing" atrophy of the cerebral cortex. There was extensive cerebellar degeneration and no evidence of vascular dysfunction, so that our patient seems to fall into the group described by Brownell and Oppenheimer. This patient serves to emphasize that neuropathological findings may change during the course of disease, and that some patients with different neuropathological findings may represent the same disease seen at different stages.

The brain tissue obtained (*6*) at surgical biopsy was immediately frozen at −70°C; it was later homogenized to a 5-percent suspension (weight : volume) in phosphate-buffered physiological saline (*p*H 7.4). A single chimpanzee, A54, was inoculated intracerebrally into the left frontal cortex with 0.2 ml and intravenously with 0.3 ml of the 5-percent homogenate. This animal was caged with two other chimpanzees of which one was inoculated intracerebrally with brain material from a Guamanian patient with amyotrophic lateral sclerosis; the other, intracerebrally with pooled urine from human kuru patients. All three chimpanzees entered the colony together and were inoculated on 20 November 1966. The animals were housed in chimpanzee quarters (*7*) with 40 other chimpanzees inoculated with human kuru, experimental chimpanzee kuru, and several other degenerative diseases of the central nervous system (*2*).

Thirteen months after inoculation, chimpanzee A54 developed a progressive fatal neurological disease (*8*). Its two cage mates remain completely well 16 months after inoculation. Chimpanzee A54 was killed by exsanguination. At necropsy, brain and visceral tissues were removed aseptically with separate instruments for each organ. Brain and visceral tissues were separately processed for histopathological study, electron microscopy, preparation of long-term explant cultures in vitro, and serial-transmission and virus-isolation studies.

No gross pathological lesions were observed in the central nervous system or organs, apart from slight cortical atrophy over the vertex of the brain. Histological study was in parallel on coded brain specimens from A54, a neurologically normal chimpanzee, and a chimpanzee having the syndrome of experimental kuru.

Preliminary pathological findings in the brain of A54 were marked status spongiosus of the cerebral gray matter, with loss of nerve cells in the cortex and proliferation and hypertrophy of astrocytes, and moderate degeneration of the cerebellum. In the cerebellum, loss of nerve cells was not particularly striking, but there was marked microglial proliferation and some gliosis. While such changes also occur in experimental kuru (*9*), there were other features not characteristic of kuru that enabled this brain to be easily distinguished from that of the animal with experimental kuru. These other features included marked proliferation of the microglia throughout the cerebral cortex, some loss of Betz giant cells in the motor cortex, and degeneration of the pontine nuclei, with neuronal loss and astrocytic reaction. In the cerebral cortex there were many large, rounded cells with pale cytoplasm often containing one ill-defined inclusion body. These cells resembled the large, pale cells seen in the brain of the human patient. The combination of clinical signs and pathological findings in the chimpanzee were so generally similar (with differences in detail) to those in the human patient that it seems very likely that animal and man were suffering from the same disease.

Other causes of neurological disease in this chimpanzee must be considered. Disease may conceivably have developed either spontaneously or by transmission by contact from an animal with experimental kuru, of which the pathology has certain similarities to that seen in A54. However, during the 5-year period of these studies, no animal has developed a neurological disorder other than those actually inoculated with tissue from kuru patients or from chimpanzees having experimental kuru. Although spontaneous neurological diseases of nonhuman primates are known, none resembling that of A54 has been described (*10*). In clinical appearance the disease in A54 bears some similarity to the syndrome of experimental kuru, but there are striking differences: somnolence, hemianopsia, and

hemiparesis were seen in this animal but have not been observed in such severity in any of the 25 chimpanzees so far affected by experimental kuru.

A 5-percent suspension of brain tissue from the affected animal has been inoculated into another chimpanzee intracerebrally and intravenously (0.2 and 0.5 ml, respectively). A portion of the original human-brain biopsy inoculum, stored at −70°C for more than 1 year, has been reinoculated into another chimpanzee by the same routes and in the same quantities as in the original experiment. Brain tissues obtained at autopsy from a second patient with a severe spongiform encephalopathy (also from W.B.M.) and from two patients with diagnoses of Creutzfeldt-Jakob disease with ataxia (from the United States) have been similarly inoculated into three chimpanzees. Several other species of primates, mice, and primary and stable cell-culture systems have been inoculated.

In summary, inoculation of brain biopsy material from a patient having Creutzfeldt-Jakob disease, with severe status spongiosus, into a chimpanzee was followed after 13 months by the appearance of a subacute, progressive, noninflammatory, degenerative brain disease. The clinical course of the disease was not unlike that in the human patient, and the neuropathological findings were remarkably similar. There is no evidence that the disease was either of spontaneous origin or transmitted by contagion from chimpanzees with kuru. We believe that Creutzfeldt-Jakob disease has been experimentally transmitted to the chimpanzee, and that the disease is caused by a transmissible agent.

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6. Patient R.R., an English male aged 59, was admitted under the care of W.B.M. at the Derbyshire Royal Infirmary on 30 May 1966 because of increasing visual disturbance and confusion. Eight weeks earlier he had first noted that objects looked small or distorted. Ophthalmologic examination at that time was normal, but his vision became increasingly disturbed, and he became confused. One week prior to admission a lumbar puncture in another hospital revealed a spinal fluid protein of 80 mg/100 ml. On admission the patient was conscious, would open eyes on command, and occasionally said "Yes." He moved his legs in response to pain. All four extremities were rigid, with arms flexed and legs extended. Irregular myoclonus was present in the right leg. The optic fundi and pupillary reactions were normal. Lumbar puncture was normal except for a spinal fluid protein of 60 mg/100 ml. After admission the patient's condition continued to deteriorate. His lower extremities became flexed. Myoclonus increased markedly, involving all limbs and head, with violent jerks about 75 times per minute. A right frontal cortical brain biopsy was performed 1 month after admission. Histopathological study of the specimen showed marked astrocytic proliferation and hypertrophy, some reduction in the number of neurons, and status spongiosus of the gray matter—findings interpreted as compatible with a diagnosis of subacute spongiform encephalopathy. After biopsy the patient remained in a vegetative state until his death 5 months later. At necropsy the brain weighed 1030 g and showed very severe cortical and cerebellar atrophy. Histologically this atrophic cortex no longer showed true status spongiosus as in the biopsy, but the gray matter throughout the cortex and basal ganglia appeared collapsed to half of its former width. The cerebellum showed extensive loss of granule cells, gliosis, and many fat-containing microglial cells. In the cerebral cortex there were unusual large cells with pale cytoplasm. Mr. R. H. Shephard performed the brain biopsy and Dr. D. L. Stevens the necropsy.
7. Of the NINDB Laboratory of Slow, Latent, and Temperate Virus Infections, Laurel, Md.
8. This male, weighing 10.6 kg, was estimated to be about 3 years old at the time of inoculation. During the following year he appeared well and gained 700 g in weight. Thirteen months after inoculation he became increasingly somnolent and inactive; he developed moderate ataxia of gait and truncal titubation. Bilateral intention tremor appeared in the upper extremities, with incoordination that was much more striking on the right: he was reluctant to use the right upper limb at all; the right leg bore weight poorly. Passive tone in the extremities was normal bilaterally, and the plantar responses were neutral. The animal ignored stimuli presented in the right visual field. Neurological and general physical examinations were otherwise normal; there was no fever. Routine studies of blood and spinal fluid during the course of the illness showed no difference from normal chimpanzees. Somnolence and lethargy gradually increased; intermittent jerking of the extremities was noted during repose. In spite of supplementary feedings and intravenous fluid the animal lost almost 2 kg of body weight. When death appeared imminent 2 months after illness was first noted, the animal was killed. Cinematographic records were filmed frequently throughout the illness; for these we thank Dr. Edward David—as well as for clinical surveillance.
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28 March 1968

Chemosensory Input and Taste Discrimination in the Blowfly

Abstract. *Simultaneous recording of behavioral responses and action potentials from single chemoreceptors in the proboscis of intact blowflies revealed that acceptance of a solution can be mediated either by input from one water receptor or from one salt receptor. Rejection of higher concentrations of salt is mediated by the same salt receptor. A difference of three impulses in the first 100 milliseconds of activity can determine whether the fly accepts or rejects the solution.*

Few studies exist in which electrophysiological events in sense organs and consequent behavioral responses of the intact animal are monitored simultaneously. Usually the two studies are conducted independently, not infrequently by different investigators working in different laboratories, and causal relations are inferred ex post facto. The difficulty of establishing a meaningful correlation is no more apparent than in studies of the chemical senses where the interpretation of taste preferences in relation to sensory input remains one of the basic unsolved problems (*1*). Exceptional opportunities for attacking this problem are presented by the blowfly *Phormia regina* Meigen because it is possible to record from individual taste receptors without in any way interfering with behavioral responses by the intact animal to various taste stimuli. This report deals with the response to sodium chloride.

One set of taste organs in the fly consists of aboral labellar hairs, each of which is equipped with five bipolar neurons. Their axons pass to the central nervous system without synapsing (*2*). Of the five receptors four have been demonstrated to be chemoreceptors. One responds to water (*3*), one to certain carbohydrates (*4*), and two to salts (*5*). The specificity of receptors and the fact that only one hair need be stimulated to elicit a complete, coordinated, behavioral response means in fact that it is possible to elicit behavior by stimulating a single receptor cell. Thus, since there is only one water cell in each hair, application of water to a single hair is equivalent to application to one receptor. Similarly, since the water cell is inhibited by certain concentrations of sugar and of salt (*3*), it is possible to stimulate a single sugar receptor or a single salt receptor by choosing the appropriate concentration.